Cranfield University

Gudrun Winkler

Occurrence and Fate of Triclosan and Tetracycline in Full Scale Wastewater Treatment Plants

School of Industrial and Manufacturing Science

MSc by Research Thesis

CRANFIELD UNIVERSITY

SCHOOL OF INDUSTRIAL AND MANUFACTURING SCIENCE

MSC BY RESEARCH THESIS

Academic Year 2004-2005

GUDRUN WINKLER

Occurrence and Fate of Triclosan and Tetracycline in Full Scale Wastewater Treatment Plants

Supervisors: Elise Cartmell
Roland Fischer *(TU Dresden)*
Peter Krebs *(TU Dresden)*
Andrew Thompson
Sponsor: Paul Griffin, Severn Trent Water

October 2005

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science by Research.

Abstract

Pharmaceutical residues gain importance as they are emitted in a vast array and quantity into the aquatic environment. The main source for most pharmaceuticals are the discharges of wastewater treatment plants. Within this study four WTPs were selected with different biological treatment proccesses, such as rotating bilogical contactor (RBC), trickling filter (TF), activated sludge plant (ASP) and oxidation ditch (OD). Samples were taken each possible treatment step and analysed for their content of Triclosan and Tetracycline and selected biomass and wastewater characteristics were also determined.

Tetracycline could not be detected in any of the wastewater treatment plants, nor its degradation products, which might be suggested to their chelating nature with divalent cations, such as calcium and metals. Triclosan has been detected in almost every liquid sample of the wastewater treatment in concentrations up to 5,000 ngL^{-1} for influents and 800 ngL^{-1} for effluents.

In principle it is problematic to compare elimination rates for different wastewater treatment plants, as their influent conditions may often show wide variations. However, loss rates for Triclosan within the four different treatment systems varied between 81% and 96%, showing the best and most consistent elimination rate for the oxidation ditch.

Although Triclosan removal rates are shown to be significant high, discharges still contained substantial residual concentrations, which would therefore require further elimination steps. Triclosan amounts bound to extracellular polymeric substances seemed to adversely affect discharge values. This is most likely due to the desorption processes occurring in the secondary clarifier.

The correlation between liquid and biomass characteristics are weak to moderate, probably due to influences of uncontrolled factors associated with the operation of a full scale treatment work system from which all the samples were obtained. Among all of the determined liquid and biomass characteristics temperature, pH and lipid content showed themselves to be the most significant, with regards to the overall removal of Triclosan. Furthermore, few parameters, such as chemical oxyen demand or specific oxygen demand* within influent or oxidation ditch samples seemed to have impact on either Triclosan concentration of the bulk phase of the oxidation ditch or the Triclosan uptake within the EPS.

Acknowledgement

I would like to express my gratitude to all those who made it possible to complete this thesis. I would like to thank Dr. Elise Cartmell for offering me the chance to participate in this very interesting project and would like to express my gratitude for all her support, even beyond the project. Thanks as well to Andrew Thompson for letting me participate in his PhD-project and for giving me the chance to work so individually.

I want to thank Prof. Peter Krebs for overseeing this project on the part of Dresden University of Technology and also PD Dr. Roland Fischer, to whom I am deeply indebted to for keeping an eye on the progress of my work and always being available when I needed his advice.

I would like to express my thanks to the project sponsor, Severn Trent and Paul Griffin, and all the site personnel for always being so helpful. Furthermore, I want to thank Dr. Hilmar Börnick for giving me permission to conduct additional HPLC analysis at Dresden University of Technology and Dr. Bernd Schreiber for devoting his time to EPS-extract analysis.

Thanks to everybody in the Water Science department of Cranfield University for contributing to such a pleasant working environment. Thanks to the technicians, Rukhsana Ormesher and Jane Hubble for being so helpful all the time. Special thanks to Emma Goslan and Ray Butler for running the IC and to Jane Hubble for making TOC measurements possible.

Many thanks to Peter "Pita" West for helping out with important equipment and to Meike "Zicke" Heurich for all her support and her cheerful presence. Thanks as well to Emma "Sunshine" Sharp for struggling so bravely with the corrections of this thesis. I would really like to say a BIG thank-you to all my friends for making times so *wunderbar*, for all the support and all the cheering up. All of you made the time worth living in the countrysides of Cranfield!

Last but not least I would like to express my deepest gratitude to my family, my parents and my prospective-parents-in-law, my brothers for all their warm support in every matter. I would like to emphasise my inexpressible thankfulness to my fiancé Ruschmuba "Marko" for stepping back from his own plans to enable the writing of my thesis abroad, for his devoted support and for simply making my life so cheerful.

Contents

List of Figures

List of Tables

List of Abbreviations

2,8/2,7-DCDD	2,8/2,7-dichlorodibenzo-p-dioxin
AS	activated sludge
ASE	accelerated solvent extraction
ASP	activated sludge plant
CAFO's	confined animal feeding operation
CAS	conventional activated sludge
COC	colloidal organic carbon
COD	chemical oxygen demand
COD/DOC	specific oxygen demand
CTC	chlortetracycline
C_{EPS}	extracellular carbohydrates
C_{SMP}	soluble (microbial products) carbohydrates
c_S	concentration - sorbed on suspended solids
c_W	concentration - water phase
dm	dry matter
DOC	dissolved organic carbon
DWF	dry weather flow
E. coli	Escherichiae coli bacteria
EC_{50}	effect concentration - 50%
EDCs	endocrine disrupting compounds
EPS	extracellular polymeric substances
f_{oc}	fraction organic carbon of the suspended solids
FabI	fatty acid biosynthesis I
FQs	fluoroquinoles
HLB	hydrophilic-lipophilic-balance
HPLC	high pressure/performance liquid chromatography
HRT	hydraulic retention time
IC	inorganic carbon
K_d	partition coefficient (soil/water adsorption)
K_{oc}	organic carbon coefficient
LOD	limit of detection

LOQ	limit of quantification
log K_{ow}	log octanol-water coefficient
MBR	membrane bioreactor
OD	oxidation ditch
OTC	oxytetracycline
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PCDD	polychlorinated dibenzo-p-dioxins
PE	population equivalent
P_{EPS}	extracellular proteins
pK_a	acid - base ionisation constant
POC	particulate organic carbon
PPCPs	pharmaceutical and personal care products
P_{SMP}	soluble (microbial products) proteins
P_{VP}	vapour pressure
RAS	returned activated sludge
RBC	rotating biological contactor
SCOD	Soluble COD
SMP	soluble microbial products
SPE	solid phase extraction
SS	suspended solids
TC	total carbon
TCs	tetracyclines
TCOD	total COD
TF	trickling filter
TOC	total organic carbon
TS	total solids
UV	ultra-violet
VSS	volatile suspended solids
VTS	volatile total solids
WTP*(s)*	wastewater treatment plant*(s)*

1 Introduction

Pharmaceuticals and personal care products (PPCPs) and their metabolites have become one of the emerging issues in environmental chemistry in relation to concern about effects both to wildlife and humans. Recent studies indicate the potential widespread occurrence of low-level concentrations (ngL^{-1} to μgL^{-1}) of pharmaceuticals, hormones, and other organic sewage contaminants and their metabolites in the aquatic environment (Halling-Sørensen *et al.*, 1998; Daughton and Ternes, 1999; Kolpin *et al.*, 2002; Boyd *et al.*, 2003; Siegrist *et al.*, 2003). PPCPs and their metabolites are continually introduced into aquatic environment and are prevalent at concentrations (Kolpin *et al.*, 2002), which can affect water quality and ecosystem health and potentially impact drinking water quality (Stan and Heberer, 1997; Heberer, 2002; Heberer *et al.*, 2002).

Of particular concern is also the speculation that the prevalence of pharmaceuticals in the environment may be leading to subtle but hitherto unrecognised effects or undetected effects causing irreversible damage to the ecosystem (Daughton and Ternes, 1999). Endocrine disrupting compounds (EDCs), for instance, have been introduced into the environment in unnatural high quantities leading to adverse effects on the hormonal systems of invertebrates and vertebrates, such as decreasing fertility, deformations as well as metabolism and behavioural disturbances (Colborn and Clement, 2004). Another example for the impact of pharmaceutical in the environment, is the recently documented linkage between the use of the anti-inflammatory diclofenac in livestock treatment and the catastrophic decline in vulture population in Pakistan and India (Oaks *et al.*, 2004). Diclofenac residues in cattle cadavers have been found to evoke visceral gout in vultures, a condition caused by renal failure, leading to an endangered population decline of up to 95% of the oriental white-backed vulture *Gyps bengalensis* and of the *Gyps indicus* and *Gyps tenuirostris* since the early 1990s.

Most pharmaceutical reach the environment through the aquatic water courses. Pharmaceuticals that have been detected directly or as their metabolites include antibiotics (such as erythromycin, sulfamethoyazole, trimethropim and ciprofloaxin), analgesic and anti-inflammatory drugs (such as diclofenac, ibuprofen, acetylsalicylic acid, naproxen, ketoprofen (paracetamol)), lipid regulators (such as clofibric acid, bezafibrate, and gemfibrozil), β-blockers (such as bisoprolol, betaxolol, nadolol, metoprolol and propanolol), antiepileptic drugs (such as carbamazepine), antineoplastics (such as the oxazaphosphorines, ifosfamide

and cyclophosphamide), oral contraceptives (such as 17α-ethynyl estradiol and mestranol), and steroids and hormones (such as cis-androsterone, coprostanol, 17a-estradiol, 17β-estradiol, estrone, progesterone, and testosterone). Besides pharmaceuticals, personal care products entering the environment is another major cause for concern. Personal Care Products are defined as chemicals having intended direct end uses primarily on the human body such as fragrances (musks), preservatives (parabens), disinfectants/antiseptics (triclosan), sunscreen agents (methylbenzylidene camphor), nutraceuticals/herbal remedies (Ternes, 1998; Halling-Sørensen *et al.*, 1998; Daughton and Ternes, 1999; Kolpin *et al.*, 2002).

Little is so far known about the behaviour of pharmaceuticals in the environment and their impact on organisms. Some PPCPs entering the environment may not cause any acute adverse effect on organisms themselves, but their metabolism products might be more toxic and persistent than the parent compound or bioaccumulation might cause subtle changes. With regard to the high variety of different pharmaceuticals, considerable combination effects might occur, even if the toxicity of the single substance is negligible (Cleuvers, 2003). Antibiotics and antibacterial agents are of special interest as they are known to select bacterial resistance in human pathogens and therefore may represent a direct threat to public health (Austin *et al.*, 1999).

Wastewater treatment effluent and discharges are to be considered the main source of pharmaceuticals to enter the wider environment. Therefore, it is of great importance to understand the fate of pharmaceuticals within wastewater treatment processes and to find possible ways to reduce their release into the environment.

2 Literature Review

2.1 Pharmaceuticals in the Aquatic Environment

One of the first ever and by now most commonly detected residue of pharmaceuticals in the aquatic environment is clofibric acid, the main active metabolite of the blood lipid regulators clofibrate, erofibrate and theofibrate used in human medical care. Garrison *et al.* (1976) isolated this compound using the GS/MS-method from the effluent of the Big Blue River sewage treatment plant over 28 years ago. Since then, the occurrence of clofibric acid has been reported in several surface waters, and even in the North Sea in concentrations up to 7,8 ngL^{-1} in the plume of the river Elbe, Germany (Stan *et al.*, 1999; Stan and Heberer, 1997; Heberer, 2002; Heberer *et al.*, 2002). Clofibric acid was also the first pharmaceutical ever to have been detected in tap water. In 1993, this pharmacologically active metabolite was found in all tap water samples from the Berlin area at varying concentrations up to 165 ngL^{-1} (Stan and Heberer, 1997). Further investigations demonstrated the direct correlation between bank filtration, artificial groundwater enrichment, and the level of drinking water contamination by clofibric acid and later by several polar organic compounds which appeared at trace-level concentrations in tap water (Heberer *et al.*, 1997; Webb *et al.*, 2003). This aroused concern because of the obvious contamination of drinking water due to incomplete removal from wastewaters. In several studies from the Berlin area Heberer (2002) and Heberer *et al.* (2002) demonstrated the impact of sewage discharges on the surface water quality and finally on the drinking water contamination of large conurbations, such as Berlin.

This provided proof that not anthropogenic micro-pollutants entering the environment from wastewater treatment works could impact drinking water supplies. Several other pharmaceutical compounds, such as diclofenac, ibuprofen, phenanzene and carbamazepine, have been found in a cycle from human application via human excretions, municipal wastewater treatment plants, surface waters, groundwater recharge, and back to human drinking water (Ternes, 2001; Heberer, 2002; Heberer *et al.*, 2002). Figure 2.1 shows the possible pathway of pharmaceuticals into the environment.

In the last three decades, various groups of researchers, mainly in Europe and North America, determined different PPCP residues in surface water, groundwater, influent and effluent

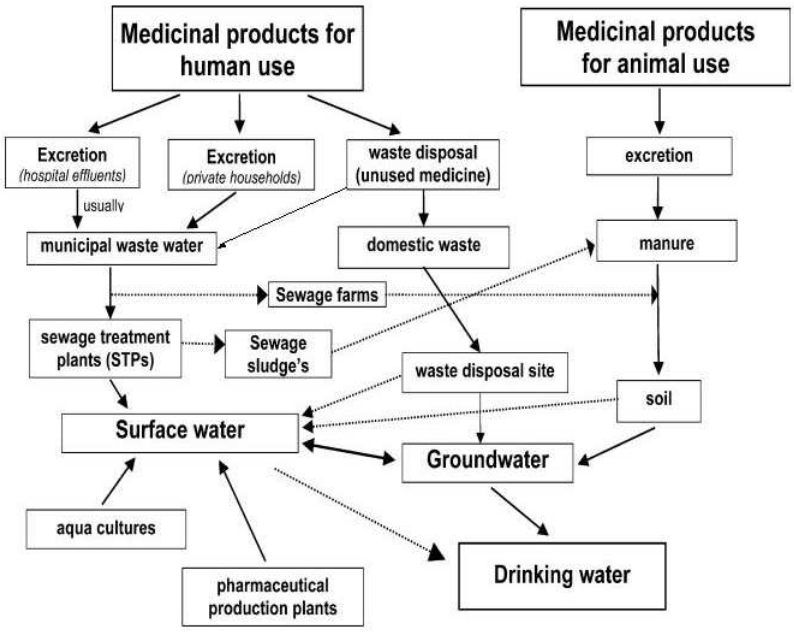


Figure 2.1: Pathways of pharmaceuticals in the environment (Heberer, 2002)

of wastewater treatment plants (WTPs), soils, sludge and as well as in plants and wildlife, especially aquatic wildlife as they are subject of primary exposure to the WTPs effluents and landfill or agriculture runoff and leaching (Garrison *et al.*, 1976; Miyazaki *et al.*, 1984; Stumpf *et al.*, 1996; Daughton and Ternes, 1999; Ternes, 1998; Thiele-Bruhn, 2003; Diaz-Cruz *et al.*, 2003; Witte *et al.*, 1989; Tolls, 2001; Stan and Heberer, 1997). Kolpin *et al.* (2002) published an investigation of organic wastewater contaminants (OWCs) in U.S. streams in 1999-2000, in which they measured concentrations of 95 selected organic wastewater contaminants in over 139 streams across 30 U.S. states. In 80% of the streams organic wastewater contaminants were prevalent and 82 out of 92 being found in this study representing a wide variety of sources including residential, industrial, and agricultural uses and origins.

Approximately 100,000 different chemicals are registered in the European Union (EU) of which some 30,000 are distributed on the market in quantities in excess of one ton. It is estimated that today about 3,300 different substances are being used as human medicaments in the EU (Giger *et al.*, 2003). About 50,000 drugs were registered in Germany for human use, 2,700 of which accounted for 90% of the total consumption and which, in turn, contained about 900 different active substances (Glaeske, 1998). In the UK approximately 3,000 active substances are licensed (Ayscough *et al.*, 2000).

It is practically impossible to assess the extent of loads of pharmaceuticals and personal care products and their metabolites which may find their way into watercourses, as their

consumption numbers are in most of the cases not regulated and controlled. The amounts of pharmaceuticals by prescription can be evaluated by multiplying the amount of daily dose with the number of prescribed daily dose per year, but there are also a large group of so called '*Over-the-counter'-products* which are purchased in pharmacies, drug stores and supermarkets in unknown quantities, e.g. mostly painkillers such as ibuprofen, paracetamol (Daughton and Ternes, 1999). A lot of personal care products containing anti-microbial agents, for instance triclosan, are distributed without any control and therefore the entry into the environment is difficult to estimate. Many of the more commonly applied drug groups, e.g. antibiotics, are used in quantities similar to those of many agrochemicals and other organic micropollutants but they are not required to undergo the same level of testing for possible environmental effects (Daughton and Ternes, 1999). Usually those pharmaceuticals in surface water are in very low concentrations, but it should be taken into consideration that long term exposure can pose a serious environmental risk. Many of PPCPs on the market are pinpointed with the adverse side effects which should be considered while consumption but it is not known how the original target effects and especially these adverse side effects might react once released into the environment. Research has shown that many such compounds can enter the environment, disperse and persist to a greater extend than first anticipated (Kolpin *et al.*, 2002).

Attention should be particularly paid to antibiotics and to antibacterial agents as it is still unclear if the prevalence of antibiotics in natural waters increases the risk of developing resistance and, what is even worse, multi-resistance amongst human potential pathogens.

2.2 Antibiotics and Antibacterial Agents - An Introduction

Antibiotics and antibacterial agents are an important group of chemical compounds with a broad spectrum of applications. They are used as antiseptic and antimicrobial agents in consumer products, in human medical care as medical treatment and also in veterinary treatment products.

In human medicine antibiotics are prescribed to treat bacterial infections and diseases, in consumer products there are mostly used as disinfectants. In veterinary treatment they are used as therapy for infections and illness as well, but most consumption occurs as permanent feed additives, as stimulation products to accelerate growth, so called '*growth promoters*' and as preventive treatments to avoid infections.

The term antibiotics (*anti-, biotikos (Greek) = belonging to the living*) was created in 1941 by Selman Waksman. Besides Prontosil, a sulfonamide antibiotic, penicillin was one of the first ever administered antibiotics. Since the mass production of penicillin in 1943, lots of various medicines with antibiotic effects have been developed and antibiotics belong to one

of the most consumed group of pharmaceuticals. The Fédération Européenne de la Santé Animal (FEDESA 2001) estimated that the total amount of antibiotics used in 1999 in the EU (incl. Switzerland, excl. new states of 2004) was 13,216 tons of which 8,528 tons were used in human therapy and 4,688 tons were applied in the veterinary sector.

The growing concern regarding the extensive use of antibiotics and antibacterial agents is the fear that resistance to antibiotics is increasing and, in the worst case scenario, multi-resistance of bacteria against antibiotics and antibacterial agents may be developed (Austin *et al.*, 1999). It is known that applications of antibiotics depending on their target mechanism of action, their applied amounts and their prescription patterns, induces in due time resistance in bacterial strains as a natural reaction (Franklin and Snow, 1998; McBain *et al.*, 2002). So, for instance, the development of resistance to fluoroquinoles antibiotics (FQs) typically occurs within 2 years of its widespread application in veterinary medicine (Endtz *et al.*, 1991). That is the reason why antibiotics used for veterinary treatment are usually not applied in human medicine and vice verse, in order to avoid possible selection for resistance (Onken, 1985). Antibiotic residues in the environment are also thought to evoke resistance in bacterial strains causing a serious threat to public health as more and more infections can no longer be treated with presently known antidotes (Franklin and Snow, 1998; Austin *et al.*, 1999).

There is therefore a need to investigate the occurrence and fate of antibiotics and antibacterial agents and the ways their emission into the wider environment can be reduced.

2.2.1 Mechanism of Action

Antibiotics and antimicrobial agents are a wide group of different compounds all of which aim to inhibit bacteria by either bacteriostatic effects (growth inhibitor) or bactericides effects (inhibition of essential metabolisms and therefore '*killing*' of bacteria). They are classified into several criteria, regarding either the mechanism of action (bacteriostatic/bactericide), the fields of application (middle-, small-, and broad spectrum) or, foremost, regarding the physiochemical criteria.

Taking just the two compounds of this survey, Tetracycline and Triclosan, into account, the antibiotic classification according the family of compounds for Tetracycline include ß-Lactam-antibiotics (penicillines), tetracyclines, aminoglykosides, makrolides-antibiotics, lincosamide, gyrase-inhibitor, sulfonamide and trimethoprim, glykopeptide-antibiotics, polypeptide-antibiotics, nitroimidazol-derivatives, while Triclosan can be seen as belonging to the group of antiseptic phenols.

General drugs are absorbed by the organism after intake and are subject to metabolic reactions, such as hydroxylation, cleavage or glucoronation adding functional groups to

the molecule (Phase I metabolites) or involve covalent conjugation to polar molecules to make the molecule more hydrophobic and better excretable (Phase II metabolites) (Forth *et al.*, 1992). Those metabolites are generally more water soluble than the parent compound. However, a significant amount of the original substance will leave the organism unmetabolised via urine and faeces (Forth *et al.*, 1992; Onken, 1985) .

2.2.2 Occurrence of Antibiotics and Antibacterial Agents in the Environment

Antibiotics and antibacterial agents may, like other pharmaceuticals, enter the environment through various pathways (*see Figure 2.1*). Beside diffuse entry of antibiotics used in veterinary medicine, wastewater treatment plants are seen as the most important sink of pharmaceuticals for the aquatic environment.

Antibiotics used in veterinary medicine are likely to enter the aquatic environment through leaching from fields, either as runoff or as percolation through the soil to the groundwater after the exposure of manure on fields as fertiliser or irrigation, or due to immense usage in livestock farms. Some spare antibiotic concentration may enter wastewater treatment works as a result of the methods used to clean out, for instance, confined animal feeding operations (CAFO's). Additionally antibiotics are used extensively in aquaculture either as feed additives or are simply distributed to the water directly.

Antibiotics used in human medicine can be found in wastewaters, derived from natural excretion or improper disposal via the toilet. However, this improper disposal seem to be of minor importance as many of the pharmaceuticals applied in human medical care are not completely eliminated in the human body (Forth *et al.*, 1992; Heberer, 2002). Some antibiotic compounds are administered as cutaneous application, which may lead to a direct wash-off without prior metabolisation. Antibacterial agents used in consumer products are also known to enter the sewer systems as '*down-the-drain products*'.

Several research groups carried out studies on the occurrence of antibiotics in the aquatic environment. Macrolide antibiotics (clarithromycin, dehydro-erythromycin [metabolite of erythromycin], roxithromycin, lincomycim), sulfonamides (sulfamethoxazole, sulfadimethoyine, sulfamethazine, and sulfathiazole), fluoroquinoles (ciprofloxacin, norfloxacin, and enrofloxacin), chloramphenicol, tylosin and trimethoprim have been found up to the low μg/l-level in sewage and surface samples (Hartmann *et al.*, 1998; Hirsch *et al.*, 1999; Lindsey *et al.*, 2001; Kolpin *et al.*, 2002; Golet *et al.*, 2001; Giger *et al.*, 2003; Reverté *et al.*, 2003; Christian *et al.*, 2003; McArdell *et al.*, 2003). Table 2.1 gives an overview of the detected concentration of certain antibiotics in surface and groundwaters.

Table 2.1: Determination of different antimicrobial agents in surface and groundwaters

Compound	**Country**	**Concentrations (ngL^{-1})**	**Reference**
	fluoroquinole antibacterial agents		
ciprofloxacin, norfloxacin	Glatt-River, (Switzerland)	< 19	Giger *et al.* (2003)
ciprofloxacin, norfloxacin	Glatt-River, (Switzerland)	< 19	Golet *et al.* (2001)
enroflaxin (ENR), ciproflaxin (CPR)	wells (Spain)	n.d.	Reverté *et al.* (2003)
	macrolides		
clarithromycin	Glatt-River, (Switzerland)	up to 79	McArdell *et al.* (2003)
erythromycin	surface waters (Germany)	up to 130-300	Christian *et al.* (2003)
erythromycin-H_2O	groundwater (Germany)	up to 49	Sacher *et al.* (2001)
erythromycin-H_2O	surface waters (Germany)	150 (med) 1700 (max)	Hirsch *et al.* (1999)
tylosin	surface water (USA)	40 (med) 280 (max)	Kolpin *et al.* (2002)
	sulfonamides		
sulfadimethoxidine	surface water (USA)	60-150	Lindsey *et al.* (2001)
sulfamethazine	surface waters (USA)	220	Lindsey *et al.* (2001)
sulfamethazine/ sulfamethoxacole	groundwater (Germany) close to wastewater irrigation area/agricultural area resp.	470 (SMX) 160 (SMT	Hirsch *et al.* (1999)
sulfamethoxacole	groundwater (Germany)	up to 410	Sacher *et al.* (2001)
sulfamethoxacole	surface waters (Germany)	up to 410	Christian *et al.* (2003)
sulfamethoxacole	surface waters (USA)	1020	Lindsey *et al.* (2001)
sulfamethoxacole	groundwater (USA)	220	Lindsey *et al.* (2001)
sulfathiazole	surface waters (USA)	80	Lindsey *et al.* (2001)
different sulfonamides	groundwater, landfill downgradient (Denmark)	very high concentrations, up to 5000	Holm *et al.* (1995)

It has been shown by Christian *et al.* (2003) that the detected concentrations of antibiotics in the environment may well reflect their amount and method of prescription (oral or cutaneous).

Extreme values of concentrations of antibiotics entering the environment can be found in some places where, in particular, production of those compounds takes place. So Holm *et al.* (1995) analysed the groundwater downgradient of a landfill formerly used for the disposal of waste from pharmaceutical production and determined a large variety of sulfonamide concentrations ranging up to 5 mgL^{-1}. In the WTP effluents of an oxytetracycline production facility in China, Qiting and Xiheng (1988) displayed an average concentration of oxytetracycline (OTC) of about 50 mgL^{-1}.

Nevertheless, these examples represent just a spot discharging extreme values of antibiotics into the environment affecting a limited area, whereas the main intake results from permanent use in medication. One starting-point to control the emission of PPCPs into the environment is to fully understand the processes of their fate and possible way of elimination in wastewater treatment works.

2.2.3 Antibiotics and Antibacterial Agents in Wastewater Treatment Plants

The behaviour and fate of antibiotics and antibacterial agents once in sewer systems and wastewater treatment plants is not well known. During wastewater treatment, pharmaceuticals can display three different types of behaviour:

1. transformation (bio-, photo-) or mineralisation
2. sorption to sewage sludge(s)
3. remain dissolved in treated effluents

The proportion of a PPCP that is removed from the wastewater effluent due to transformation or by adsorption to sludge or solids depends to a great extent on its chemical structure and physico-chemical properties, but also on the specific conditions within the respective plant. Water temperature, residence times (corresponding to flow rates), dilution with rainwater and sludge age (and thus adaptation of microbial communities) were found to exert an effect on elimination efficiencies (Buerge *et al.*, 2003; Ternes *et al.*, 1999). Studies concerning the pharmaceutical residues in the environment have clearly shown that elimination in municipal wastewater treatment plants is often incomplete (Ternes, 1998). This was not only suggested by the fact that active compounds are often not fully eliminated, but can also enter wastewater plants mostly conjugated to polar molecules (e.g as glucoronides). Those conjugates can be cleaved during wastewater treatment and finally the unchanged compounds discharge into the aquatic environment through the WTPs effluents (Stan and Heberer, 1997; Halling-Sørensen *et al.*, 1998; Ternes, 1998; Ternes *et al.*, 1999; Daughton and Ternes, 1999; Kolpin *et al.*, 2002).

Golet *et al.* (2001) and Giger *et al.* (2003) detected fluoroquinoles (ciprofloxacin, norfloxacin) in several wastewater treatment effluents at concentrations ranging up to 108 ngL^{-1}. Fluoroquinoles (FQs) excrete largely unchanged (generally <25% metabolized). Fluoroqunioles (FQ) mass flow reduction resulted in 88-92% during wastewater treatment, mainly due to sorption on sewage sludge and no significant removal of FQs occurred under methanogenic conditions of the sludge digester.

McArdell *et al.* (2003) found erythromycin concentrations up to 330 ngL^{-1} in WTP effluents. Worth noting is the fact that the concentration of one WTP depended highly on seasonable periods, and was found to be twice as high in the winter period than in the summer period. It is interesting to note that significantly higher effluent concentrations in the WTP, serving Switzerland's most important international airport, were thought to be a result of higher consumption pattern of erythromycin in other European countries (4-50 times higher than in Switzerland).

Miao *et al.* (2004) investigated the final effluents of eight WTPs, located in five Canadian cities for 31 antimicrobials from different groups including macrolide, quinolone, quinoxaline dioxide, sulfonamide and Tetracycline. Ciprofloxacin, clarithromycin, erythromycin-H_2O, ofloxacin, sulfamethoxazole, sulfapyridine, and Tetracycline were frequently detected in the effluents.

Some literature data are given in Table 2.2.

Table 2.2: Concentration of different antibiotics in WTP effluents

Compound	**Type of WTP**	**Concentrations (ngL^{-1})** - final effluent (tertiary, secondary resp.)	**Country**	**Reference**
		fluoroquinole antibacterial agents		
ciprofloxacin, norfloxacin	different WTP	36 - 106	Switzerland	Giger *et al.* (2003)
ciprofloxacin	WTP (of 5 cities)	118 (med) 400 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
norfloxacin	WTP (of 5 cities)	500 (med) 1120 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
ofloxacin	WTP (of 5 cities)	94 (med) 506 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
ciprofloxacin	WTP (of 4 cities)	45 - 108	Switzerland	Golet *et al.* (2001)
norfloxacin	WTP (of 4 cities)	48 - 120	Switzerland	Golet *et al.* (2001)
ofloxacin	WTP	330 (med) 580 (max)	France, Greece, Italy, Sweden	Andreozzi *et al.* (2003)

Table 2.2: (continued)

Compound	**Type of WTP**	**Concentrations (ngL^{-1})** - final effluent (tertiary, secondary resp.)	**Country**	**Reference**
ciprofloxacin, norfloxacin	WTP	60 (med) 80 (max)	France, Greece, Italy, Sweden	Andreozzi *et al.* (2003)
trimetroprim	WTP	40 (med) 130 (max)	France, Greece, Italy, Sweden	Andreozzi *et al.* (2003)
ciprofloxacin	hospital effluent	3000 (med) 8700 (max)	Switzerland	Hartmann *et al.* (1998)
		macrolides		
clarithromycin	WTP	57 - 330	Switzerland	McArdell *et al.* (2003)
erythromycin-H_20	WTP	2500 (med) 6000 (max)	Germany	Hirsch *et al.* (1999)
erythromycin-H_20	WTP	110 - 200	Switzerland	McArdell *et al.* (2003)
roxythromycin	WTP	11 - 31	Switzerland	McArdell *et al.* (2003)
clarithromycin	WTP (of 5 cities)	87 (med) 536 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
erythromycin-H_20	WTP (of 5 cities)	80 (med) 838 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
roxythromycin	WTP (of 5 cities)	8 (med) 18 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
		sulfonamides		
sulfamethoxazole	WTP	50 (med) 90 (max)	France, Greece, Italy, Sweden	Andreozzi *et al.* (2003)
sulfaacetamide	WTP (of 5 cities)	64 (med) 151 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
sulfamethazine	WTP (of 5 cities)	363 (med) 363 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
sulfamethoxacole	WTP (of 5 cities)	243 (med) 871 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
sulfapyridine	WTP (of 5 cities)	81 (med) 228 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
sulfatdiazine	WTP (of 5 cities)	19 (med) 19 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
sulfisoxacole	WTP (of 5 cities)	19 (med) 34 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)

Within wastewater treatment the action and behaviour of antibiotics and antibacterial agents is of great interest, as their original aim of inhibiting the action of bacteria could have negative effects on biological treatment steps (Halling-Sørensen, 2001). Although

the concentration of antimicrobial agents found in wastewater are generally at least 100-times less than the concentrations necessary to inhibit the growth of resistant bacteria, the concentrations can affect susceptible bacteria (Kümmerer *et al.*, 2000) and have the potential to determine a selection in favour of resistant bacteria (Guardabassi *et al.*, 2002).

Another major issue of concern is the fact that WTPs are considered to be a sink for antibiotic resistant bacteria, entering the aquatic environment and able to spread their drug resistance by, e.g. gene transfer (Franklin and Snow, 1998). The contamination of river water by antibiotic resistant bacteria such as *Escherichia coli* and coliform group bacteria from wastewater treatment effluents has been proved by several research groups (Young, 1993; Goni-Urriza *et al.*, 2000; Iwane *et al.*, 2001; Guardabassi *et al.*, 2002).

However, even though indicating the effluents of WTPs as a source of antibiotic resistant bacteria for the contamination of the aquatic environment, wastewater treatment processes themselves showed a decline of antibiotic resistant bacteria in most cases (Iwane *et al.*, 2001; Guardabassi *et al.*, 2002). This was, indeed, in contrast to other studies, which indicated an increase in the prevalence of resistant bacteria following conventional wastewater treatment (Bell *et al.*, 1983).

These contradictory insights may well be the result of different plant operations, varying examination targets, such as bacterial population, antimicrobial and antibiotic agents, as well as the bacteriological methods and the breakpoint values used to determine antimicrobial resistance (Guardabassi *et al.*, 2002). This shows the difficulties of comparing such complex connections in wastewater treatment processes.

2.2.4 Antibiotics and Antibacterial Agents in Sludge & Soils

Treated sewage sludges are commonly used in Europe as a fertiliser for agriculture. For this reason the effects of pharmaceuticals on the efficiency of treatment stabilisation processes, as well as the fate of re-used sludge have to be investigated further.

On the one hand, antibiotic and antibacterial agents are thought to interfere with anaerobic or aerobic sludge digestion, and might lead to a decrease of the stabilisation efficiency resulting, in the worst case, in insufficiently treated sludge with antibiotic residues and the possibility of selection for antibacterial resistant strains. Lallai *et al.* (2002) determined the effects of three antibiotics, amoxicillin trihydrate, oxytetracycline hydrochloride and thiamphenicol on the anaerobic digestion of sewage sludges. While oxytetracycline did not show significant inhibition of the methanogenic bacteria, the other two tested compounds seemed to have slight adverse effects. Sanz *et al.* (1996) concluded that antibiotics have an adverse effect on anaerobic digestion and methane production according to their mechanism of action. A study concerning the selection for increasing bacterial resistance by

Guardabassi *et al.* (2002) concluded that sludge digestion caused rather a reduction in the total number of bacteria resistant to any of the 14 tested antimicrobial agents and rather than any significant increase in the prevalence of resistant bacteria.

On the other hand, antibiotic residues in the treated sludges or liquid manure, once exposed on soils or sludge disposal sites, may leach through to the soil and interact with microorganisms in the soil. Antibiotic residues are thought to percolate through the soil into groundwater gradients or be washed-off into surface waters. In particular, the polar nature of several pharmaceuticals can lead to leaching from sludge treated soils into groundwater or runoff into surface waters (Alder *et al.*, 2001). Some of the excreted metabolites can even be transformed back to the original active drug. So, for instance, the glucoronide of chloramphenicol or N-4-acetylated sulfamethazine were reported to have converted to chloramphenicol and sulfamethazine, respectively, in liquid manner (Berger *et al.*, 1986).

Table 2.3 provides some literature data of concentrations of fluoroquinole antibiotics. The detected concentrations of fluoroquinoles (ciprofloxacin, norfloxacin) in sewage sludges from several WTPs ranged from 1.4 to 2.4 $mgkg^{-1}$ of dry matter (dm). Norfloxacin and ciprofloxacin were antibiotic compounds which have been found to be sorbed relatively well to suspended solids (Golet *et al.*, 2001), but showed to be persistent within soils as well, even after long-term observation.

While within the European Community debates are taking place about conducting intensive further studies regarding the persistence of pharmaceuticals in sediments, soils and sludges after the disposal of sewage sludge and manure as fertiliser and their '*Predicted No Effect Concentration*', Switzerland has taken a more rigorous decision and has banned the disposal of sewage sludge into agriculture since January 2003.

Table 2.3: Concentration of antibiotics in different types of sewage sludges and soils

Compound	**Type of sludge**	**Description of sample**	**Concentration (10^3 ngkg^{-1}dm)** in sludge/ sediments	**Country**	**Reference**
		fluoroquinole antibacterial agents			
ciprofloxacin	sewage sludge	untreated (raw) sludge	1,400-2,000	Switzerland	Golet *et al.* (2001)
ciprofloxacin	sewage sludge	digested sludge	2,270-2,420	Switzerland	Golet *et al.* (2001)
ciprofloxacin	sewage sludge	sludge treated soil (8 month after application)	350-400	Switzerland	Golet *et al.* (2001)
ciprofloxacin	sewage sludge	sludge treated soil (12 month after application)	270-280	Switzerland	Golet *et al.* (2001)
ciprofloxacin, norfloxacin	sewage sludge	-	1,400-2,400	Switzerland	Golet *et al.* (2001)
norfloxacin	sewage sludge	untreated (raw) sludge	1,540-1,960	Switzerland	Golet *et al.* (2001)
norfloxacin	sewage sludge	digested sludge	2,130-2,370	Switzerland	Golet *et al.* (2001)
norfloxacin	sewage sludge	sludge treated soil (8 month after application)	290-320	Switzerland	Golet *et al.* (2001)
norfloxacin	sewage sludge	sludge treated soil (12 month after application)	270-300	Switzerland	Golet *et al.* (2001)

2.2.5 Environmental Concern

Antibiotics and antibacterial agents are designed to affect mainly microorganisms according to their physico-chemical properties. Though having a specific mode of action, they can also have numerous effects on non-target species even in low concentrations. Those low concentrations might not have a direct acute effect, but due to bioaccumulation subtle changes can develop.

Antibiotics and antibacterial agents have been reported to bioaccumulate in aquatic organisms, such as fish and waterplants (Miyazaki *et al.*, 1984; Kuch *et al.*, 2003) . For some pharmaceutical compounds bioaccumulation has been connected to estrogenic activity and

shown impact on sexual differentiation in fish (Purdom *et al.*, 1994; Jobling *et al.*, 1996; Routledge *et al.*, 1998). These high adverse effects have not been shown so far for antibiotics and antibacterial agents, but weak androgen or estrogen activity had to be admitted in conducted tests (Ishibashi *et al.*, 2003; Foran *et al.*, 2000).

Concerns have been raised regarding public health issues over the occurrence of antibiotics in the environment (Halling-Sørensen *et al.*, 1998) as well as by identification of increased bacterial resistance in waste effluent from hospitals and pharmaceutical plants (Goni-Urriza *et al.*, 2000). Miranda and Zemelman (2001) detected resistant bacteria even in wild fish the Bay of Concepción, Chile, a highly commercial fishing ground, and suggested an influence of wastewater effluents on the varying resistant strains. However, there is no evidence so far for the increase of antibiotic resistance caused by the prevalence of antibiotics in discharges rather than by the excretion of resistant microorganisms by man and animals and the spread of resistance by plasmid transfer. The incline of antibiotic resistant bacteria found in healthy people in several countries in the last number of decades have been found to show evidence between the consumption of antibiotics and the increasing amount of multiresitant bacteria (Austin *et al.*, 1999).

Most pharmaceuticals are subject to possible transformation/degradation processes resulting in various metabolites in the environment. Those metabolites can have greater or lesser physiologic activity than the parent compounds. They can be toxic themselves or be the base for further transformation resulting in toxic compounds. Some pharmaceuticals which are not or only slightly persistent might result in higher persistent metabolites. The occurrence of metabolites has generally not been studied, but should be investigated further to understand fully the fate and transport of pharmaceuticals in the environment.

2.2.6 Summary

Unlike pesticides/agrochemicals which are applied sporadically, antibiotics are being continually released to the environment via wastewater treatment charges (WTPs) or even untreated discharges to the rivers (Daughton and Ternes, 1999) and due to the run-off and leachate from sludges and manure recycled onto soils (Alder *et al.*, 2001; Heberer, 2002). In comparison to conventional organic contaminants, such as pesticides, PAHs, and PCBs, there is little or no information available on the fate and transformation (bio- and phototransformation) of antibiotics in soil or water. Besides the reduced application of antibacterial agents and antibiotics, an increase of removal in wastewater treatment processes provides the best opportunity to avoid environmental contaminations. Little is known so far about the fate of these compounds in wastewater treatment plants.

Therefore an ample need exists for further investigations for the occurrence and fate of

antibiotics and antibacterial agents and their environmental risk assessment and the possibilities to avoid or reduce their emission into the environment.

2.3 Tetracycline

Tetracyclines are an extremely important group of antibiotics in widespread use in both human and veterinary medicine, and as growth additives in animal feed, having a bacteriostatic, broad spectrum against gram-positive and gram-negative bacteria (Onken, 1985). Tetracyclines have been widely used in confined animal feeding operations (CAFOs) for meat, milk, and fish production. For instance, Yang and Carlson (2004) cited that Tetracycline and oxytetracycline are currently two of the ten approved antibiotic growth promoters in the USA. Bacterial resistance is known to develop readily, which has severely limited its use in recent years (Speer *et al.*, 1992; Klein and Cunha, 1995).

2.3.1 Structure of Tetracyclines and Tetracyclines metabolites

Tetracyclines Tetracyclines are chemically characterised by a partially conjugate four-ring group with a carboxyamide functional group. They are amphoteric compounds as characterised by three pK_avalues. Depending on the alignment of the additional groups they are divided into several groups (Figure 2.2). Chlortetracycline (CTC), oxytetracycline and Tetracycline (TC) are representative members of this antibiotic class (Onken, 1985; Foran *et al.*, 2000).

Besides natural tetracyclines isolated from various strains of streptomyces many derivatives (e.g. doxycycline, minocycline) have been prepared by their chemical conversion (Franklin and Snow, 1998). Chlortetracycline (CTC), oxytetracycline (OTC) and Tetracycline (TC) are prepared by fermentation with specific streptmoyces strains and by adding precursors (Onken, 1985). Tetracyclines distinguish due less to their antibacterial activity than due to their pharmacocinetics properties. They can be administered both orally and parenterally. For further details see *Appendix 2.3.1* for chemical and structural information of the three tetracyclines (CTC, OTC, TC).

Tetracycline Tetracycline (TC) has a melting point of 172,5°C, a $\log K_{ow}$ of -1.33 and is known to have poor stability under strong acid and alkaline conditions with reversible formation of epimers at position C4 to 4-epitetracycline (ETC) in a weak acid (pH 3) and to anhydro-Tetracycline under strong acidic conditions (below pH 2) (Zhu *et al.*, 2001; Oka *et al.*, 1989). Tetracycline are rapidly metabolised and moreover form relatively stable complexes with divalent cations, e.g. calcium, magnesium and with metal cations (Vartanian

et al., 1998). Oka *et al.* (1989) reported the photodecomposition of Tetracycline in aqueous solutions similar to those in fish ponds.

The major metabolites of Tetracycline (TC), 4-epitetracycline, anhydrotetracycline and 4-epi-anhydrotetracycline are given in Figure 2.3. Anhydrotetracycline is a toxic decomposition of Tetracycline and the toxic side effect has been attributed to the conformational changes in the ring system (dos Santos *et al.*, 1997).

2.3.2 Mechanism of Action

Tetracyclines are broad-antibiotics. In particular, they are used on intracellular pathogens such as rickettsiae (typhoid fever), spirochetes (syphilis) and mycoplasmas. Intracellular pathogens have the ability to penetrate somatic cells and to proliferate within the cells. Tetracyclines dock onto the special binding site of the ribosome and therefore interrupt the ribosomal protein synthesis of intracellular bacteria and consequently, inhibit the reproduction of the bacteria (Onken, 1985; Franklin and Snow, 1998).

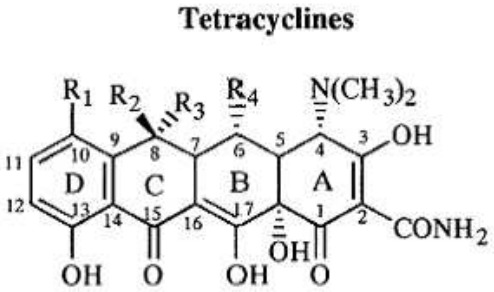
Bacterial resistance against Tetracycline is ubiquitous and due to the fact of similar structure, cross-resistance among the several groups are given, which leads to an emasculate use for therapy of this group (Onken, 1985). As with most pharmaceutical compounds tetracyclines may be excreted unchanged after oral administration (*see Table* 2.4).

Table 2.4: Excretion rate of tetracyclines and metabolites Hirsch *et al.* (1999)

Compound	**Prescribed doses 1995, Germany (Mio.)**	**Excretion rate (%)**		
		unchanged	glucoronides	other metabolites
chlortetracycline	1.9	> 70	-	-
tetracycline	-	80-90	-	-
minocycline	8	~60	-	~40
oxytetracycline	-	>80	-	-
doxycycline	80.2	> 70	-	-

2.3.3 Occurrence of Tetracyclines in the Environment

Tetracyclines (TCs) have a high potential to enter the environment due to excess use in herd treatment and aquaculture (Boxall *et al.*, 2003). TCs readily chelate with divalent ions, such as calcium, magnesium and metals, forming a complex which reduces their bioavailability,



	R_1	R_2	R_3	R_4
Oxytetracycline(OTC)	H	CH_3	OH	OH
Chlortetracycline(CTC)	Cl	CH_3	OH	H
Demeclocycline(DMC)	Cl	OH	H	H
Tetracycline(TC)	H	CH_3	OH	H
Doxycycline(DXC)	H	CH_3	H	OH
Minocycline (MNC)	$(CH_3)_2N$	H	H	H
Melocycline (MCC)	Cl	-	CH_2	OH

Figure 2.2: Tetracycline classification (Onken, 1985)

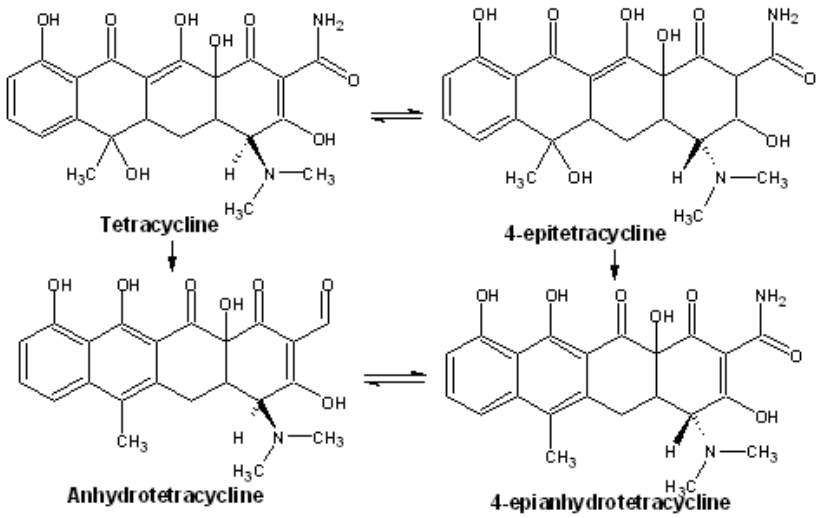


Figure 2.3: Tetracycline and its major metabolites, 4-epitetracycline, anhydrotetracycline, and 4-epi-anhydrotetracycline

and causes them to partition readily into the biomass (Halling-Sørensen *et al.*, 2002). TCs also bind to humic acids and proteins, especially via anionic functional groups (Loke *et al.*, 2002).

Christian *et al.* (2003) determined antibiotics in German surface and groundwaters. Tetracyclines (chlortetracycline, doxycycline, oxytetracycline, and Tetracycline) could not be detected within this study. This is in agreement with the results of other studies which have shown that tetracyclines can sorb strongly to soil organic matter and mineral particles and are therefore rarely found in the free form in surface waters (Christian *et al.*, 2003; Tolls, 2001; Hirsch *et al.*, 1999; Samuelsen *et al.*, 1992; Daughton and Ternes, 1999; Hamscher *et al.*, 2002).

However, Chlortetracycline, oxytetracycline, and Tetracycline were detected in very low frequencies (1.2 - 2.4%) in U.S.-surface streams (Kolpin *et al.*, 2002) using one analytical method and were not detected with another analytical method. Another study by Lindsey *et al.* (2001) also detected tetracyclines in U.S.-surface waters. Yang and Carlson (2004) detected several tetracyclines in the Poudre River (Colorado, U.S.A) with no other significant tributaries than point-sources from wastewater effluents and non-point sources from agriculture. They concluded that most of those compounds are only present in the river when there are agricultural influences, as a result that is consistent with their extensive use in as animal feed additives.

Literature data of the detected concentrations are given in Table 2.5.

Table 2.5: Concentration of tetracyclines in different surface and groundwaters

Compound	Country	Concentrations (ngL^{-1})	Reference
Chlortetracycline	U.S. - stream surface waters	420 (med) 690 (max)	Kolpin *et al.* (2002)
	surface water samples (USA)	150	Lindsey *et al.* (2001)
Democlocycline	surface water samples (USA)	110 (min) 325 (max)	Yang and Carlson (2004)
Doxycycline	U.S. - stream surface waters	n.d.	Kolpin *et al.* (2002)
Oxytetracycline	U.S. - stream surface waters	340 (med) 340 (max)	Kolpin *et al.* (2002)
	surface water samples (USA)	40 (min) 1,340 (max)	Lindsey *et al.* (2001)
Tetracycline	U.S. -stream surface waters	110 (med) 110 (max)	Kolpin *et al.* (2002)
	surface water samples (USA)	110	Lindsey *et al.* (2001)
	surface water samples (USA)	50 (min) 150 (max)	Yang and Carlson (2004)

2.3.4 Occurrence of Tetracyclines in Wastewater Treatment Plants

On the basis of the extensive use of tetracyclines in human medicine and their known excretion rates, van der Heide and van de Plas (1984) calculated a maximum sum concentration of tetracyclines of 13 μgL^{-1} in WTP effluents under the assumption of 95% elimination during the treatment process.

Chlortetracycline and tetracyclines are used mainly in growth promoter for livestock. They are two of the 10 antimicrobials licensed as growth promoters for livestock in the U.S.A., explaining their detection in high concentrations in wastewater lagoons on swine farms (Zhu *et al.*, 2001; Campagnole *et al.*, 2002). Zhu *et al.* (2001) detected chlortetracycline, oxytetracycline, and Tetracycline in lagoon samples from confined animal operation waste water. 23 out of 26 samples contained over 3 μgL^{-1} of one of the three Tetracycline compounds. Concentrations were highest in lagoons for swine finishing operations where these

compounds may be routinely used for growth promotion. Maximum concentrations were for chlortetracycline 12,000 μgL^{-1}, Tetracycline and oxytetracycline up to 1,300 μgL^{-1}. Samples from groundwater degradients of those swine lagoons did not contain detectable levels of tetracyclines.

In several studies tetracyclines where not determined in sewage or natural waters, which can be attributed to the nature of TCs as described above. Interestingly, studies conducted in Europe could not detect tetracyclines either in wastewater or surface waters, whereas North-American studies detected Tetracycline in both medias. The only found literature source on overall removals from tetracyclines was the study carried out by Yang and Carlson (2004) on the Fort Collins Drake Water Reclamation Facility, Colorado, U.S.A. Unfortunately, no specific data was given about the type of wastewater treatment plant.

Literature data on the concentration of tetracyclines in wastewater plants are given in Table 2.6. The overall removal rates are given in Table 2.7.

As tetracyclines are designed to inhibit bacteria it is not surprising that Halling-Sørensen (2001) found inhibition of tetracyclines for nitrifying bacteria. The EC_{50} on *Nitrosomonas europeae* were found for Tetracycline 4.0 mgL^{-1}, Oxytetracycline 1.71 mgL^{-1} and 0.64 mgL^{-1} for Chlortetracycline. However, those high concentrations are rather to be expected in wastewater from hospitals or animal feeding farms than in conventional wastewater treatment plants. And it has to be admitted that chelating effects might lower the activity of Tetracyclines entering wastewater treatment plants.

2.3.5 Occurrence of Tetracyclines in Sludge & Soils

As mentioned above Tetracyclines are also known to bind easily with divalent ions, such as calcium, magnesium and iron and are therefore more likely to be found in sediments and sludges. Double-charged cations, like calcium occur in high concentrations in soil (Christian *et al.*, 2003).

Hamscher *et al.* (2002) determined Tetracycline residues in soil which had been fertilised with liquid manure. 4 mg kg^{-1} Tetracycline and 0.1 mg kg^{-1} (dm) chlortetracycline was detected in the distributed liquid manure. The highest concentrations in soil samples were (one year later) 86.2 (0-10cm), 198.7 (10-20cm) and 171.7 μg kg^{-1} (20-30cm) Tetracycline and 4.6-7.3 μg kg^{-1} (all three sublayers) chlortetracycline. In laboratory tests Chlortetracycline Hamscher *et al.* (2002) found out that the persistence in soil was dependant on the temperature mixed with chicken faeces with final concentration of 5.6 $mg(kg\ soil)^{-1}$: at 30°C 44% remained; at 20°C 88% and at 4°C no degradation occurred.

Tetracyclines are known to have adverse effects on sludge digestion, mostly affecting the methagonese phase. Chlortetracycline, for instance, was found to be a powerful inhibitor

Table 2.6: Tetracyclines - concentrations in wastewater effluents

Compound	**Type of WTP**	**Concentrations (ngL^{-1})** raw sewage, primary effluent, resp.	**Concentrations (ngL^{-1})** final (tertiary, secondary resp.) effluent	**Country**	**Reference**
chlortetracycline oxytetracycline tetracycline	lagoons of CAFOs	up to $1.3 \cdot 10^7$ (CTC) up to $1.3 \cdot 10^6$ (OTC, TC)	-	Nebraska (USA)	Zhu *et al.* (2001)
chlortetracycline oxytetracycline tetracycline	WTP	-	n.d. (LOD<50)	Germany	Hirsch *et al.* (1999)
chlortetracycline oxytetracycline doxycycline	WTP	240 (med) 640 (max)	-	Colorado (USA)	Yang and Carlson (2004)
doxytetracycline	WTP	-	0.038 (med) 0.046 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
tetracycline	WTP	-	0.151 (med) 0.977 (max)	Vancouver Area (Canada)	Miao *et al.* (2004)
tetracycline	WTP	980	160	Colorado (USA)	Yang and Carlson (2004)
tetracycline doxytetracycline oxytetracycline	hospital effluent	n.d. (TC) 184 (DTC) 189 (OTC)	-	Bonn (Germany)	Färber and Skutlarek (2004)
tetracyclines (sum of DTC & OTC)	WTP (approx. 300.000 PE)	32	n.d. (LOD<40)	Bonn (Germany)	Färber and Skutlarek (2004)

Table 2.7: Tetracycline overall removal in the Fort Collins Drake Water Reclamation Facility, Colorado, U.S.A. (Yang and Carlson (2004)

Compound	**Influent (ngL^{-1})**	**Effluent (ngL^{-1})**	**Overall removal (%)**
tetracycline	980	160	83.7
chlortetracycline	260	-	-
oxytetracycline	640	-	-
doxytetracycline	220	90	59.1
democlocycline	1140	90	92.1

of anaerobic digestion (Sanz *et al.*, 1996). Halling-Sørensen *et al.* (2002) reported toxicity of tetracyclines and their metabolites on bacteria in sludge and soils.

Winckler and Graffe (2000) found concentrations in liquid manure of several mgL^{-1} even after application of these drugs in recommended dosages. Considering their high persistent rate in liquid manure treated soils shows how important it is to ascertain the impact of these residues on the environment.

2.3.6 Environmental Concern of Tetracycline

Tetracyclines are applied in significant large amounts in veterinary treatment. They have been found to bioaccumulate in milk, eggs and meat from treated cattle and poultry respectively and therefore might have adverse effects on consumers. Relatively high levels in food can provoke allergic reactions in some hypersensitive individuals and transfer drug-resistant bacteria from food to humans (Wegener *et al.*, 1990).

Due to their excessive use they are likely to enter the environment and hence might have adverse effects on non-target organisms. Little is known so far about the behaviour of Tetracyclines and their metabolites in the environment. Wollenberger *et al.* (2000), for instance, showed acute chronic toxicity of antibiotics on *Daphnia magna*. Oxytetracyclines produced longer-lasting significant effects on soil microorganism (bacteria, fungi) (Thiele and Beck, 2001), while no adverse effects were found on soil fauna at environmental relevant concentrations (Baguer *et al.*, 2000).

Of significant concern is the fact that Tetracyclines evoke bacteria resistance. Reinthaler *et al.* (2003) reported up to 57% resistance to Tetracycline in *E. coli* strains in wastewater treatment plants, with hospital wastewater having higher resistance rates than municipal wastewaters. Even though the number of *E. coli* decreased within the wastewater treatment process a significant amount of resistant bacteria have been found in the discharging effluents. Those bacteria, released to the environment, are capable of transferring their resistant-plasmides to human pathogens causing multi-resistance in the worst case.

2.4 Triclosan

Triclosan is an antiseptic agent that has been widely used for almost 30 years in a vast array of consumer products. It is used as broad-spectrum bacteriostatic activity against gram-negative and gram-positive bacteria, moulds and yeast in products like soaps, skin-care lotions and creams, toothpastes (Colgate Total®), mouth rinses, deodorants, cosmetic products, shampoo, sponges, socks, under-ware, footwear (insoles of shoes, so-called *Odour-Eaters*) and recently in plastic products such as children toy's and cutting boards. It has been used in underarm deodorants and deodorant soaps since the 1960s (Jungermann, 1968) and was first introduced into the health care industry in a surgical scrub in 1972 and in toothpaste in Europe in 1985 (Jones *et al.*, 2000). In Europe, approximately 350 tons of Triclosan are presently used as an antimicrobial substance in many consumer products (Ciba Specialty Chemical, 1998).

The main manufacturer is Ciba-Geigy that uses the names Irgasan® DP300 and Irgacare® MP. Impregnated fibres and polymers have names like Ultra-Fresh®, Amicor®, Microban®, Monolith®, Bactonix® and Sanitized® (Adolfsson-Erici *et al.*, 2002). According to the European Economic Community (EEC) directive 76/768 its use is permitted at a maximum concentration of 0.3% (w/w) in consumer products.

2.4.1 Structure of Triclosan and its Metabolites

Triclosan (CAS RN 3380-34-5) is a diphenyl ether (bis-phenyl) derivate known as either 2,4,4'-Trichloro-2'-hydroxydiphenyl ether or 5-Chloro-2-2(2,4-dichlorophenoxy)phenol. It is a relatively small compound with a molecular weight of 289.5 $gmol^{-1}$, sparingly solubility in water (10 mgL^{-1} in distilled water at 20°C), hydrolytically stable, relatively non-volatile (vapor pressure [P_{VP}] = $4{\cdot}10^{-6}$mm Hg at 20°C), relatively high hydrophobicity (estimated log octanol-water partition coefficient (K_{ow}) of 4.8), stable until 280°C and a half-live in soil-degradation test of 15 to 35d (Ciba Speciality Chemicals, 2001*a*; Merck, 1983). According to Ciba Speciality Chemicals (2001*a*) Triclosan has a $pK_a$of 8.14.

Triclosan is related in its structure to a number of bis-phenyl polychlorinated and bis-phenyl chlorophenol compounds. Due principally to the synthesis chemistry of polychloro diphenyl ethers and phenoxy phenols there is the potential for the formation of small amounts of unwanted trace by-products (Menoutis and Parisi, 2001).

Structure of Triclosan is given in Figure 2.4.

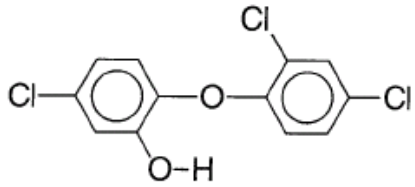


Figure 2.4: Structure of Triclosan

Triclosan is photolytic unstable and in the presence of bases, UV-light or thermical excitation it can form polychlorinated dioxins and furans and belongs therefore to the group of pre-dioxins (Nilsson *et al.*, 1974). Kanetoshi *et al.* (1988*a*); Okumura and Nishikawa (1996) demonstrated the ability of Triclosan's photolytical conversion into 2,8-dichlorodibenzo-p-dioxin (2,8-DCDD), which has been approved for aquatic systems by Latch *et al.* (2003) and Mezcua *et al.* (2004).

Higher chlorinated derivatives of Triclosan are (Kanetoshi *et al.*, 1988*a*):

- tetra II : 4,5-dichloro-2-[2,4-dichloro-phenoxy]-phenol
- tetra III : 5,6-dichloro-2-[2,4-dichloro-phenoxy]-phenol
- penta : 4,5,6-trichloro-2-[2,4-dichloro-phenoxy]-phenol

Potential photo- and biotransformation by-products are

- Methoxy-Triclosan (TCS-OMe): 5-chloro-2[2,4-dichloro-phenoxy]-anisole (Miyazaki *et al.*, 1984; Lindström *et al.*, 2002)
- 2,4 Dichlorophenol (Hundt *et al.*, 2000), which is also a synthesis compound
- 2,7/2,8-dichlorodibenzo-p-dioxin (Kanetoshi *et al.*, 1988*a*; Okumura and Nishikawa, 1996; Latch *et al.*, 2003; Mezcua *et al.*, 2004)

Structure of some Triclosan's derivatives are given in Figure 2.5 (Okumura and Nishikawa, 1996):

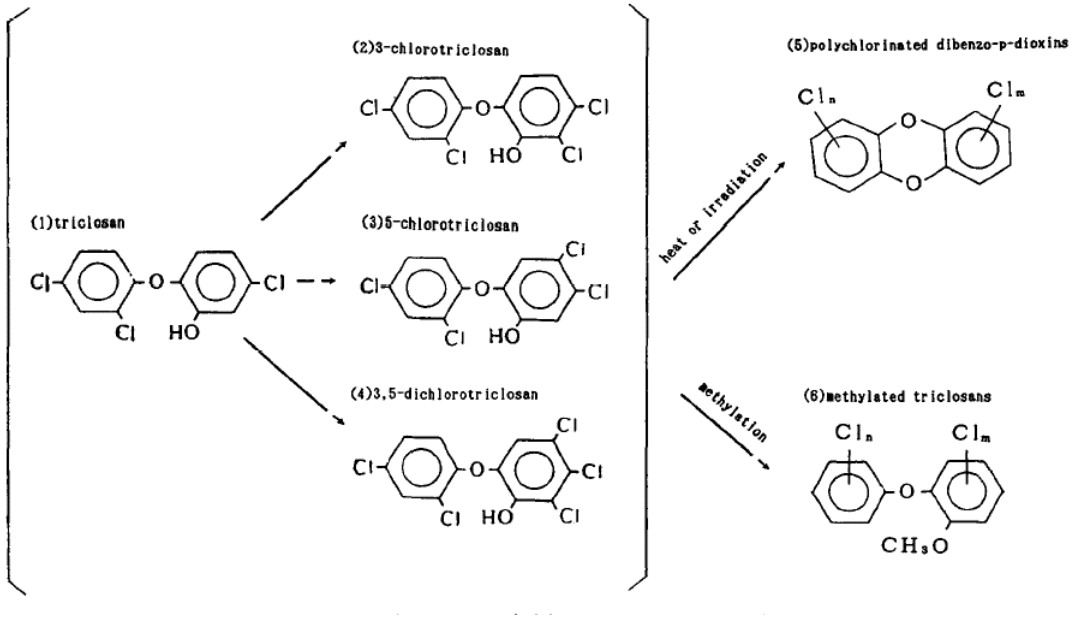


Figure 2.5: Structures of Triclosan derivatives (Okumura and Nishikawa, 1996)

2.4.2 Mechanism of Action

Triclosan is a broad-spectrum bacteriostatic used against gram-negative and gram-positive bacteria, moulds and yeast and possesses as well some antifungal and antiviral properties (Lee *et al.*, 2003). It can be counted to the class of bisphenols, a class of compounds that exhibit a broad spectrum of antimicrobial activity. Another formerly widely used member of this group was hexachlorophene (2,2'-dihydroxy-3,5,6,3',5,6'-hexachlorodiphenylmethane), which has been limited in consumer products due to toxic concerns (de Groot *et al.*, 1994) and was replaced in most cases by Triclosan.

Triclosan mechanism of action to bacteria has been elucidated by a number of researchers (McMurry *et al.*, 1998; Levy *et al.*, 1999). Evidence suggest that Triclosan inhibits effectively the enzyme enoyl-[acyl carrier protein] reductase (FabI), which is involved in the bacterial lipid biosynthesis (McMurry *et al.*, 1998). However, *Streptococcus pneumonia* is highly sensitive to Triclosan and does not contain a FabI homolog, while *Pseudomonas aeruginosa*, which is highly resistant to Triclosan does contain FabI (Heath and Rock, 2000).

2.4.3 Occurrence of Triclosan in the Environment

Due to its wide use Triclosan is an ubiquitous compound in the environment. It has been detected in various studies in the aquatic environment (Miyazaki *et al.*, 1984; Kolpin *et al.*, 2002; Lindström *et al.*, 2002; Agüera *et al.*, 2003; Singer *et al.*, 2002; Bester, 2003; Balmer *et al.*, 2004) and has even been found in human milk (Adolfsson-Erici *et al.*, 2002). In a monitory study of 139 streams in the United States, Triclosan has been one of the most frequently detected compounds (Kolpin *et al.*, 2002)

Miyazaki *et al.* (1984) detected Triclosan residues and its methylated derivatives in Tama River and Tokyo Bay in Japan and the methylated form accumulated in fish and shellfish. Similar studies have been carried out recently by Balmer *et al.* (2004) and Singer *et al.* (2002) in Swiss lakes and fish. Regarding bioaccumulation, Schettgen (2000) and Orvos *et al.* (2002) found that Triclosan is more likely to be bioaccumulated within lower pH and the methylated form is more likely to bioaccumulate than Triclosan itself (Schettgen, 2000). Kuch *et al.* (2003) detected Triclosan in water plants of discharge receiving rivers in concentrations between 84 μg(kg dm)$^{-1}$ and 1403 μg(kg dm)$^{-1}$. This indicates a high trend to bioaccumulation of this antibacterial agent.

Boyd *et al.* (2004) accomplished a 6 months study for several compounds including Triclosan in stormwater canals, which are connected to the lake Pontchartain (New Orleans, USA). Triclosan has been found in concentrations up to 29 ngL^{-1} in the canals and the lake median concentration exhibited 4.6 ngL^{-1}. This decrease was attributed to possible removal and degradation processes. Singer *et al.* (2002) also observed a high overall removal rate of 0.03 d^{-1} in the epilimnion of lake Greifensee (Switzerland) due to photochemical reactions.

Morrall *et al.* (2003) detected relatively low Triclosan concentrations upstream in a creek (34 ngL^{-1}) and 431 ngL^{-1} 200 m from effluent outfall, and finally a concentration of 104 ngL^{-1} 8,000 m from outfall. This rates corresponded to a 76% reduction of Triclosan and the overall loss of in river water exhibited similar loss to BOD.

Regarding the stated loss of Triclosan due to sunlight, where no degradation products were the subject of study, the conclusions of Latch *et al.* (2003); Mezcua *et al.* (2004) give rise to concern. Kanetoshi *et al.* (1988*a*,*b*); Okumura and Nishikawa (1996) stated already the possibility of Triclosan's conversion into 2,8-dichlorodibenzo-p-dioxin (2,8-DCDD), which has been approved for aquatic systems by Latch *et al.* (2003) who found that Triclosan can convert easily under sunlight in surface water into that kind of dioxin, whereas Mezcua *et al.* (2004) found 2,7/2,8-DCDD in wastewater.

Table 2.8 gives an overview of detected Triclosan concentrations in surface waters.

Table 2.8: Literature data of Triclosan concentration in surface waters

Compound	Country	Concentrations (ngL^{-1})	Reference
Triclosan	U.S. - stream surface waters	140 (med) 2,400 (max)	Kolpin *et al.* (2002)
	lake water (Louisiana, USA)	4.6 (med) 14.9 (max)	Boyd *et al.* (2004)
	river water (UK)	19	Sabaliunas *et al.* (2003)
	greek water (TX, USA)	34	Morrall *et al.* (2003)
	greek water (TX, USA)	431 (115 min*) 223 (585 min*) 104 (1485 min*)	Morrall *et al.* (2003)
	surface water (Switzerland)	4 (med) 14 (max)	Lindström *et al.* (2002)
Methyl-Triclosan	surface water (Switzerland)	16 (min) 33 (max)	Lindström *et al.* (2002)

*time corresponded to hydraulic flow after discharge points

2.4.4 Occurrence of Triclosan in Wastewater Treatment Plants

Wastewater treatment plants are to be considered the main source of this *'down-the drain'* compound for the environment. High variations can be expected in sewer systems and sewage influents and, according to the overall removal of the specific wastewater treatment, as well in the WTP effluents. Indeed, several studies found Triclosan in high concentrations in wastewater influents as well as in effluents. See Table 2.9 for some selected literature data.

Possible paths for Triclosan loss in wastewater treatment processes are sorption (either to sewer biofilms, suspended solids or biomass), settling processes or transformation processes, such as biodegradation or phototransformation. Losses due to stripping by aeration are negligible, as this compound is relatively non-volatile (vapor pressure $[P_{VP}] = 4{\cdot}10^{-6}$mm Hg at 20°C) and lipophilic.

According to **biodegradation** tests like OECD 301C (MITI I) or OECD 302C (MIT II) (1993) Triclosan is not readily or inherently biodegradable. This might be in the first instance expectable, as Triclosan aims at the inhibition of bacteria, but is more likely to be a result of the highly used concentrations and therefore being in toxic dosage to bacteria. This is in accordance to Orvos *et al.* (2002), where Triclosan showed only toxic effects on WTP microorganism at concentrations greatly exceeding those expected in WTPs influents.

Federle *et al.* (2002) conducted a study for batch activated sludge test and continuous activated sludge (CAS) in relatively low concentrations, relevant for conventional wastewater treatment works. Triclosan has been found to be readily biodegradable under these conditions, showing overall removal rates of 94.7% (at $10\mu gL^{-1}$ influent concentration), whereas complete degradation exceeded 85.1% (79.1% mineralised and 6% incorporated into biomass). Only 5.3% of the parent compound was present in the effluent. This is similar to studies on conventional wastewater treatment works where 50% of the whole incoming Triclosan seemed to be transformed and only 5% were residues in the final effluent (Bester, 2003).

These high removal rates are a sign of the ability of adaption of microorganisms towards Triclosan. Indeed, Federle *et al.* (2002) recorded higher removal rates under shock load conditions, when the biomass had been previously exposed to Triclosan. Moreover, Orvos *et al.* (2002) suggested that the Triclosan removal might be a detoxination process. This has also been mentioned by Hay *et al.* (2001). Dokianakis *et al.* (2004) conducted a study on the inhibition of bacterial nitrite oxidation, showing that the nitrite oxidizer has the ability to overcome the inhibitory effect of Triclosan in just a few days.

Table 2.10 gives an overview of recorded literature data about overall removal rates in full scale wastewater treatment plants, ranging from 34%-96% for Activated Sludge (Singer *et al.*, 2002; McAvoy *et al.*, 2002; Sabaliunas *et al.*, 2003; Kuch *et al.*, 2003; Mezcua *et al.*, 2004), 58%-96% for Trickling Filter (McAvoy *et al.*, 2002; Sabaliunas *et al.*, 2003; Kuch *et al.*, 2003), 44%-92% (Lindström *et al.*, 2002) for different types of WTPs. These high variations in overall removal within each group of wastewater treatment process are most likely the results of the given conditions and indicates once more the difficulties in the comparison of these high complex treatment processes.

Table 2.9: Triclosan - concentrations in wastewater effluents

Type of WTP	**Concentration (ngL^{-1})** raw sewage, primary effluent, resp.	**Concentration (ngL^{-1})** final (tertiary, secondary resp.) effluent	**Country**	**Reference**
WTP	-	10 - 12	Louisiana (USA)	Boyd *et al.* (2003)
8 WTPs	-	42 (min) 213 (max)	Switzerland	Singer *et al.* (2002)
7 WTPs	910 (med) 1,300 (max)	214 (med) 650 (max)	Switzerland	Lindström *et al.* (2002)
WTP	1,600 (min) 2,600 (max)	<1000	Sweden	Pedersen and Nielsen (2003)
WTP	3,700 (min) 562,000 (max)	100 (min) 269,000 (max)	Spain	Mezcua *et al.* (2004)
WTP (different types)	300 (min) 1480 (max)	5 (min) 100 (max)	Germany	Kuch *et al.* (2003)
Activated Sludge (AS)	1,300 (min) 37,800 (max)	400 (min) 22,100 (max)	Spain	Agüera *et al.* (2003)
Activated Sludge (AS)	1,200 (med) 1,300 (max)	51 (med) 59 (max)	Germany	Bester (2003)
Activated Sludge (AS)	520 (med)	45 (med)	Switzerland	Singer *et al.* (2002)
Activated Sludge Plants (AS)	5,210 (min) 10,700 (max)	240 (min) 410 (max)	Ohio (USA)	McAvoy *et al.* (2002)
Activated Sludge (AS)	21,900	110	UK	Sabaliunas *et al.* (2003)
Activated Sludge (AS)	670	32	UK	Kanda *et al.* (2003)
Activated Sludge (AS)	1100	27	UK	Kanda *et al.* (2003)
3 Trickling Filter Plants (TF)	3,830 (min) 16,600 (max)	1,610 (min) 2,700 (max)	Ohio (USA)	McAvoy *et al.* (2002)
Trickling Filter (TF)	-	785	Texas (USA)	Morrall *et al.* (2003)
Trickling Filter (TF)	7,500	340	UK	Sabaliunas *et al.* (2003)
Trickling Filter (TF)	2,500	140	UK	Kanda *et al.* (2003)
Trickling Filter (TF)	3,700	130	UK	Kanda *et al.* (2003)
Storm Water Canals	-	15 (med) 29 (max)	Louisiana (USA)	Boyd *et al.* (2004)
7 WTPs	<1 (med) 4 (max)	4.5 (med) 11 (max)	Switzerland	Lindström *et al.* (2002)

Table 2.10: Triclosan - overall removal in different wastewater treatment plants

Type of WTP	**Overall removal of Triclosan** (%)	**Reference**
Activated Sludge (AS)	96	McAvoy *et al.* (2002)
Activated Sludge (AS)	34 (min) 69 (max)	Mezcua *et al.* (2004)
Activated Sludge (AS)	96	Bester (2003)
Activated Sludge (AS)	94	Singer *et al.* (2002)
Activated Sludge (AS)	95	Sabaliunas *et al.* (2003)
Activated Sludge (AS)	83 - 92	Kuch *et al.* (2003)
Activated Sludge (AS)	95.2	Kanda *et al.* (2003)
Activated Sludge (AS)	97.5	Kanda *et al.* (2003)
Trickling Filter (TF)	77 - 96	Kuch *et al.* (2003)
Trickling Filter (TF)	95.5	Sabaliunas *et al.* (2003)
Trickling Filter (TF)	58 - 86	McAvoy *et al.* (2002)
Trickling Filter (TF)	94.4	Kanda *et al.* (2003)
Trickling Filter (TF)	96.5	Kanda *et al.* (2003)
WTP (7 different)	44 (min) 92 (max)	Lindström *et al.* (2002)

Great importance should be paid to the already mentioned fact that **phototransformation** processes under environmental conditions might convert Triclosan into polychlorinated-dibenzodichloro-*p*-dioxins (PCDDs).

Following the study of Latch *et al.* (2003) where triclosan in aqueous media was found to convert easily under sunlight into the 2,8-dibenzodichloro-*p*-dioxin, Mezcua *et al.* (2004) identified 2,7/2,8-dibenzodichloro-*p*-dioxin as a major photolysis degradation of Triclosan in wastewater samples. Spiked wastewater samples were irradiated with natural sunlight under different pH values. Triclosan was degraded only at higher pH into dioxin, which was in accordance with the hypothesis postulated by the overlapping of the UV spectra of Triclosan and solar light only at high pH values (see Figure 5.14, page 91). Another important influence on the degradation of Triclosan into dioxin has been found to be caused by the matrix effects. Organic matter content showed a significant decrease in Triclosan concentration and an increase in the formation of 2,7/2,8-dibenzodichloro-*p*-dioxin compared to conducted experiments in reagent water.

Further field samples were analysed from an urban wastewater plant and influent and

effluent samples were determined for the two compounds. Detected concentrations are given in Table 2.11. Worth noting is the fact that the wastewater treatment process showed a high overall removal of 87.1% for 2,7/2,8-DCDD. Nevertheless, the fate of this dioxin is still unknown and it might well be that further transformation process result in higher chlorinated forms. It is not necessary to emphasise the need for further studies.

Table 2.11: Triclosan and 2,7/2,8-DCDD in a wastewater treatment plant

Triclosan			**2,7/2,8-Dichlorodibenzo-*p*-dioxin**		
Influent* (10^3ngL^{-1})	Effluent* (10^3ngL^{-1})	Overall removal (%)	Influent* (10^3ngL^{-1})	Effluent* (10^3ngL^{-1})	Overall removal (%)
2.3 (min)	0.1 (min)	36.9 (min)	0.02 (min)	0.004 (min)	80.0 (min)
562.0 (max)	369.0 (max)	95.7 (max)	8.9 (max)	0.4 (max)	96.9 (max)
106.1 (med)	58.9 (med)	72.0 (med)	3.2 (med)	0.2 (med)	87.1 (med)

*data from Mezcua *et al.* (2004)

2.4.5 Occurrence of Triclosan in Sludge & Solis

According to other discussed antibiotics and antibacterial agents Triclosan, which has been sorbed to sludge or biomass during wastewater treatment process and not fully metabolised, can well be found in digestion units and may have inhibitory effects on sludge stabilisation or be released later on due to sludge re-use. Table 2.12 gives some literature data of different biomass types. Bester (2003) analysed sewage sludges of 20 wastewater treatment plants, with a concentration range of 400 - 8,800 μg kg^{-1} per day in different sludge samples. The concentration pattern within those samples represented well the influences of capita. The relatively high amounts found in digested sludge (McAvoy *et al.*, 2002) and the fact that Triclosan is not effectively removed during anaerobic sludge digestion, leads to concern regarding its possible agricultural use, even though Triclosan is expected to be be rapidly biodegradable in soil environment, with determined half-lives ranging from 15-35d (Ciba Speciality Chemicals, 2001*a*).

Being released into the environment due to effluent discharges, the remaining Triclosan is as well likely to be sorbed to the biomass matrix, such as sediments and soils. Singer *et al.* (2002) analysed a screen of vertical concentration profile in a sediment core of the lake Greifensee (Switzerland) reflecting the increasing usage of Triclosan over the past 30 years,

based on the assumption that no degradation happened in the lakes sediments. The later point has not been verified so far, but the quite high amount of Triclosan in the depth profile of the 30 year old sediment core showed that the degradation has to be very low. Kuch *et al.* (2003) stated similar results for a determined sediment core of the lake Bodensee (Germany). Agüera *et al.* (2003) stated the sorption of Triclosan in marine sediments at wastewater discharge point.

Mezcua *et al.* (2004) have not found 2,7/2,8-dibenzodichloro-*p*-dioxin in particulate matter samples from a filter of the WTP plant, whereas Triclosan was detected. This was suggested to the fact that the dioxin displays more affinity towards water and is unlikely to be sorbed to solids. This statement can, however, not be in accordance to the structure of dioxins and hence their chemical behaviour, which is known to show very high lipophility. However, Mezcua *et al.* (2004) stated further that no fortifications were carried out on the type of biomass and it might also be the case that the extraction of the dioxin was not efficient enough or levels were lower than the detection limits (Mezcua *et al.*, 2004).

Table 2.12: Triclosan - literature data on sludge/sediment concentration

Compound	**Type of WTP**	**Concentration in sludge/sediments** ($ngg^{-1}dm$)	**Country**	**Reference**
Triclosan	Activated Sludge (AS)	1,200	Germany	Bester (2003)
	Activated Sludge Plants	PS: 8,750-14,700 SS: 900-4,200	Ohio (USA)	McAvoy *et al.* (2002)
	Trickling Filter Plants (TF)	PS: 8,750-14,700 SS: 900-4,210 DS: $5.3 \cdot 10^5$-$1.56 \cdot 10^7$	Ohio (USA)	McAvoy *et al.* (2002)
	Marine Sediments	0.27-130.0	Spain	Agüera *et al.* (2003)

PS - primary sludge; SS - secondary sludge; DS - digested sludge

2.4.6 Environmental concern of Triclosan

According to Ciba Speciality Chemicals (2001*a*) Triclosan has been extensively tested for human safety and at concentrations used in consumer products it is not acutely toxic, nor is it carcinogenic, teratogenic, or irrigating to eyes and skin. It has not yet been shown to be toxic to mammals, but is has been found to have adverse effects on aquatic organisms such as algae, daphniae, tadpoles and fish at relevant concentrations (Orvos *et al.*, 2002; Adolfsson-Erici *et al.*, 2002; Wilson *et al.*, 2003; Fraker and Smith, 2004). Very little is known about the subtle effects in long term exposure to (aquatic) organisms.

Triclosan itself is thought to select for antibacterial resistance (McMurry *et al.*, 1998; Schweitzer, 2001), including multiple antibiotic resistance and to evoke possible endocrine disrupting effects (Foran *et al.*, 2000; Ishibashi *et al.*, 2003) due to its similar structure to non-steroidal estrogens. Triclosan also causes concern with regard to its pre-dioxin properties (Kanetoshi *et al.*, 1988*a*,*b*; Latch *et al.*, 2003; Mezcua *et al.*, 2004). Kanetoshi *et al.* (1988*a*) found Triclosan as an important source of PCDDs in the environment by conducting out tests with textile fabrics under exposure to sunlight, while Latch *et al.* (2003) and Mezcua *et al.* (2004) showed its conversion for aquatic media under environmental conditions.

Ishibashi *et al.* (2003) found high toxicity in the early life stage of medaka *Oryzias latipes* and that the metabolite of triclosan might be a weak estrogenic compound with the potential to induce vitellogemin in male medaka but with no adverse effect on reproductive success and offspring. Triclosan's estrogenic effects could not be supported by Foran *et al.* (2000) but a possible potential as a weak androgenic compound had to be admitted.

The selection of antibacterial cross-resistance directly due to the use of Triclosan in household products or in hospitals could not be, in principle, verified (Al-Doori *et al.*, 2003; Cole *et al.*, 2003). Nevertheless, Triclosan adapted *Escherichia coli* demonstrated resistance to antibiotics (Levy *et al.*, 1999; Braoudaki and Hilton, 2004*b*). Similar results have been reported for *Pseudomonas aeruginosa* and *Salmonella entericae* (Chuanchuen *et al.*, 2001; Braoudaki and Hilton, 2004*a*). Hay *et al.* (2001) found that Triclosan could be used by a consortium of bacteria as sole source of carbon and energy and it has been suggested that Triclosan degradation process may be a detoxification process, while Hundt *et al.* (2000) reported a degradation of Triclosan by *Trametes versicolor* and *Pycnoporus cinnabarinus*.

2.5 Micropollutants in Wastewater Treatment Plants

The fate and behaviour of micropollutants, such as PPCPs, within wastewater treatment becomes an emerging issue as they have shown to aversely impact the environment and thus representing a possible thread to human health.

The fate and behaviour of micropollutants in wastewater treatment is not well known, yet. As previously discussed micropollutants are not entirely removed within wastewater treatment and were found even in high concentrations in discharging effluents.

The proportion of micropollutants which are removed from the wastewater effluent due to transformation or by adsorption to sludge or solids depends to a great extent on their chemical structure and physico-chemical properties, but also on the specific conditions within the respective plant. Water temperature, residence times (corresponding to flow rates), dilution with rainwater and sludge age (and thus adaptation of microbial communities) were found to exert an effect on elimination efficiencies (Ternes *et al.*, 1999; Buerge *et al.*, 2003; Ternes *et al.*, 2004).

As removal due to stripping can be necleted for most PPCPs, the main removal mechanisms are therefore:

1. transformation (bio-, photo-) or mineralisation
2. sorption to sewage sludge(s)

Compounds which do not exhibit to react to these mechanisms may remain dissolved in high quantities in the treated effluents.

Reducing the input of micropollutants into the environment, requires therefore to fully understand the fate and behaviour of micropollutants within wastewater treatment in order to enhance their removal from wastewater discharging effluents.

The aim of this study was to determine the impact of selected wastewater and biomass characteristics on the removal of micropollutants in different full scale wastewater treatment plants with different biological treatment stages, such as fixed bed biofilms and activated sludge systems.

2.5.1 Biological Treatment

Biological treatment involves the use of microorganisms to break down compounds, which are regarded as environmental contaminants. The biological treatment aims to transform products into those which are considered to be non-pollutants or to sorb these compounds to the remaining sludges. In the beginning of the last century the first invention was a

conventional fixed-culture procedures of the bacterial bed type (bacterial filter, biological disk) and has been developed further with various type of sludge treatment systems. The most conventional treatment system nowadays are aerated tanks, so called activated sludge tanks.

Elimination of trace substances within wastewater treatment plants depends mainly on the level of development in the purification stage. The most important steps are *sorption to suspended solids* (where substances are eliminated through settling processes within primary, secondary treatment steps), *decomposition of substances through bacteria* (biological mineralisation or transformation within the biological treatment step of the wastewater treatment plant) and *stripping through aeration* (which is negligible within this study as Triclosan is of low volatility).

The transformation or **decomposition** of compounds can take place under aerobic, anoxic or anaerobic conditions. As the previously discussed micropollutants mostly occur in wastewater, in concentration ranges from 10^{-5} - 10^{-9} gL^{-1}, biological degradation is only possible where the bacteria have primary substrate available (Bouwer, 1989; Siegrist *et al.*, 2003). A distinction is made between *co-metabolism*, where bacteria only partly break down or convert the compound and do not use it as carbon source and *mixed substrate growth*, in which the substance is used as a carbon and energy source and hence the bacteria totally mineralised it (Siegrist *et al.*, 2003). In the case of Triclosan, for instance, total mineralisation has been recorded in activated sludge systems (Federle *et al.*, 2002; Bester, 2003), whereas anaerobic conditions showed no significant decomposition for Triclosan (McAvoy *et al.*, 2002). Hay *et al.* (2001) observed the use of Triclosan as carbon source for bacterial growth, which suggests that Triclosan can be used as primary carbon source. Similar results have been observed by Bouwer (1989), where chlorophenols were shown to be used as primary substrate in biofilms.

The interactions among environmental factors, such as dissolved oxygen, oxidation-reduction potential, temperature, pH, availability of nutrients, salinity, particulate matter, competing organisms, and concentrations of compounds and organisms, often control whether biotransformation can occur.

According to the report of the POSEIDON project (Ternes *et al.*, 2004), the biological degradation of PPCPs within wastewater treatment can be predicted by pseudo first order kinetics. The equations for plug flow or batch reactor and for cascade compartments are given below.

For a plug flow or a batch reactor:

$$\frac{C_{i,out}}{C_{i,in}} = e^{-k_{i,biol} \cdot SS \cdot HRT} = e^{-k_{i,biol} \cdot SP \cdot SA} \tag{2.1}$$

where :

$C_{i,in}$ influent substance concentration of the compound i $[\mu g L^{-1}]$
$C_{i,out}$ final substance concentration of the compound i $[\mu g L^{-1}]$
$k_{i,biolog}$ kinetic constant for pseudo first order degradation $[L\ gSS^{-1}\ d^{-1}]$
HRT hydraulic retention time of the whole reactor or duration of the batch $[d]$
SP specific sludge production per amount of wastewater treated $[gSS\ m^{-3}_{wastewater}]$
SP specific sludge production per amount of wastewater treated $[d]$

In the case of a cascade of completely stirred reactors of equal volume, the relative concentration in the effluent can be calculated from the following equation (Ternes *et al.*, 2004).

Cascade with **n** *numbers of compartments:*

$$\frac{C_{i,out}}{C_{i,in}} = \left(\frac{1}{1 + k_{i,biol} \cdot \frac{SS \cdot HRT}{n}}\right)^n = \left(\frac{1}{1 + k_{i,biol} \cdot \frac{SP \cdot SA}{n}}\right)^n \tag{2.2}$$

with:

n number of cascade compartments [–]

The reaction rate constant $k_{i,biolog}$ $[\mu g L^{-1}]$ has to be determined within laboratory batch experiments and does not only depend on the degradability of each specific compound, but also on the sludge decomposition (Ternes *et al.*, 2004):

k_{biol}<0.1 : no substantial removal due to biological degradation

0.1<k_{biol}<10 : degree of removal strongly dependent on reactor configuration

k_{biol}>10 : more than 95% of removal by biological degradation

Besides the above mentioned effects of biodegradation, sorption capacity of biofilms and activated sludges have been known to play an important role in elimination processes (McAvoy *et al.*, 2002).

Sorption of compounds is usually divided into adsorption and absorption. Possible sorption sites are extracellular polymeric substances (EPS), cell wall, cytoplasmic membrane, or cytoplasmic membrane (Flemming, 1995). *Adsorption* describes the attachment of substances to the surface or the boundary surface between the solvent and the adsorbent, while *absorption* describes the process where the substance diffuses into the absorbent and

therefore passes into another phase. In most cases it is hard to define between adsorption and absorption and therefore these processes are generally described as *sorption* (Weber *et al.*, 1991).

Sorption of xenobiotics, such as Triclosan, might aid microorganisms within the biomass to enhance the biodegradation by removing inhibitory compounds from solution, thereby reducing their toxic effects on microbial growth (Bouwer, 1989). Other compounds, such as Tetracycline, for instance are known to sorb readily with divalent cations, such as iron, calcium or magnesium, forming stable complexes and removing them therefore from the liquid phase (Halling-Sørensen *et al.*, 2002).

Results from the POSEIDON project stated that the proportion of quantities in a fully mixed tank can be predicted by the following equation (Ternes *et al.*, 2004):

Sorbed quantities in a fully mixed tank:

$$\frac{C_{i,sorbed}}{C_{i,sorbed} + C_{i,soluble}} = \frac{K_{d,i} \cdot SS}{1 + K_{d,i} \cdot SS} \tag{2.3}$$

where :

$C_{i,sorbed}$ concentration of the compound i sorbed onto the sludge $[\mu g L^{-1}]$
$C_{i,soluble}$ soluble concentration of the compound i $[\mu g L^{-1}]$
$K_{d,i}$ sorption coefficient of the compound $[L kg^{-1}]$
SS suspended solids concentration in raw wastewater or production of suspended solids in primary of secondary treatment, per L of treated wastewater $[kg L^{-1}_{wastewater}]$

This equation was followed by a simple equilibrium condition, where the concentration sorbed onto sludge *($C_{i,sorbed}$)* is assumed to be proportional to the concentration in solution *($C_{i,soluble}$)* (Ternes *et al.*, 2004):

$$C_{i,sorbed} = K_{d,i} \cdot SS \cdot C_{i,soluble} \tag{2.4}$$

It is important to note that no correlation could be found so far for the observed K_d values for sludge of municipal wastewater treatment with literature values for the specific compounds (e.g. K_{OW} or K_{OC} values). Furthermore, the relevant SS value is not the suspended solid concentration of the mixed liquor, but the amount of sludge generated per unit wastewater treated. This is due to the fact, that sludge retention time is significantly higher than the hydraulic retention time. Assuming that sludge which has been recirculated during wastewater treatment is in equilibrium with the compound concentration in solution, only newly generated sludge is available for sorption (Ternes *et al.*, 2004).

2.5.2 Sludge Extraction

When examining the fate of a compound within wastewater treatment processes it is important to know not only its path and fate within the liquid phase but also its tendency to sorb and persist in both biomass and sludge samples. Therefore preliminary work for the extraction of Triclosan and Tetracycline of biomass has been undertaken.

Extracting micropollutants from biomatrices with high amounts of organic substances usually requires a thorough sample preparation. In general, all single analysis steps can be fitted to the following simple algorithm (Figure 2.6, Antusch (1999)):

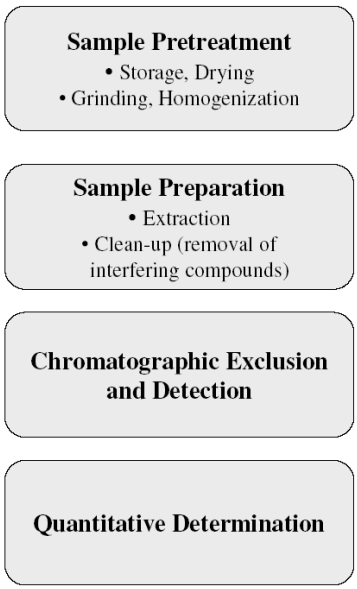


Figure 2.6: Generally algorithm for analysis of organic trace compound - sludge extraction

This algorithm may require additional steps, variations and combinations according to the type of biomatrix. The chromatographic separation and qualitative determination must be chosen in accordance with the target group of compounds. The sample pretreatment and preparation is required in oder to avoid losses of the target compound(s) and the input of possibly interfering substances.

Sample pretreatment Sample pretreatment is required before laboratory analysis. Following sample collection, the sample itself should not be subject to any changes. The storage temperature can be lowered (cool or sub-zero storage conditions) in order to reduce microbial and enzymatic conversions. The drying of samples is also a method of conservation and combines the advantage of obtaining a comparable reference value, such as dry

matter. Freeze drying proposes a gentle and conservative way of drying microbial active samples, such as sewage sludge samples (Antusch, 1999). '*The Guideline for determination of polycyclic aromatic compounds in soils and waste*' (Blankenhorn and Hornung, 1992) highlights that "concentration to dryness" at 105°C must be avoided in order to avoid significant losses of the target compounds. Previous work regarding the freeze drying of sludges showed no significant losses of trace substances (Witte *et al.*, 1989) and is therefore the most commonly used method for drying and homogenising environmental biomatrices. The determination of solid and semi-solid samples requires further grinding and homogenising in order to produce particles sizes of less then 100 μm , which could be achieved by using a zircon-oxide grinding machine to avoid any loss of the sample.

Sample preparation The aim of sample preparation is to produce a measurable solution and should be seen in combination with the available detection method(s). It is necessary to find an appropriate method to detach the target compound from the biomatrix into a measurable solution and, if necessary, to concentrate low values into detectable values.

Extraction methods There are various extraction methods, such as Soxhlet extraction, wet extraction, sonic-extraction, solid-phase extraction (SPE). Usually non-polar extraction solvents are used, such as, acetone, n-hexane, dichloromethane or iso-propanol.

Clean-up The clean up step is required for separation of unwanted extraction by-products, such as humic acids, sulfur, lipids or organic polymers, which might interfere with the detection analysis for the target compounds. The most common clean-up procedure is the gel-permeation-chromatography, silica clean-up or SPE clean-up.

2.5.2.1 Extraction Methods

There exist several literature sources about extraction methods for Tetracycline and Triclosan from biomatrices, such as soil, sewage sludge and for instance, eggs.

Table 2.13 represents few selected extraction methods from literature.

Table 2.13: Examples of extraction methods for Triclosan and Tetracycline from biomatrices

Compound	Description of biomass	Extraction method (simplified depiction)	Detection method	Reference
Sludge extraction methods for Tetracycline				
Tetracyclines (tetracyclines, OTC, CTC)	animal slurry (and also soils, environmental samples, such as egg)	liquid-liquid extraction of 1(g) with 1.2 mL of citrate buffer (pH 4.7) and extraction (twice) with 6 mL of ethyl acetate - evaporation to dryness - reconstitution with 200μL of 90% acetonitrile/10% 10 mM ammonium acetate	HPLC (separation) - microbiological assay (detection) / MS-MS with LCQ ion trap	Sczesny *et al.* (2003)
Tetracyclines	soil	1g of wet sludge - vortex with 1.2mL 1 M citrate buffer(pH 4.7) - 6 mL of ethyl acetate - vortex and 15min shaker - evaporation to dryness - reconstitution in 90% acetonitrile, 10% mM ammonium acetate in water	LC-MS-MS	Hamscher *et al.* (2005)
Sludge extraction methods for Triclosan				
Triclosan	sludge, sediments	lyophilised sample (1g) - dichloromethane, Dionex ASE 200 (accelerated solvent extraction) - nitrogen blow down to 2mL - clean up over silica extraction cartridges - transfer into toluene by rotary evoparator - clean up with SPE, silica based - nitrogen	GC-MS	Singer *et al.* (2002)
Triclosan	sewer biofilms	lyophilisation - Soxhlet-extraction hexane/acetone (2:1, 16 h) - gelpermeation chromatography - silica gel (hexane-toluene)	GC-MS	Antusch (1999)
Triclosan, methyl-Triclosan, Tetra II, Tetra III, Penta	sewage sludge	lyophilised sample (0.5g) - supercritical fluid extraction module (CO2) with silica gel - n-hexane elution - nitrogen blow down to 0.2mL - derivatisized with N,N-diethyltrimethylsilylamine	GC-MS	McAvoy *et al.* (2002)

3 Aims & Objectives

Wastewater Treatment Plants (WTPs) are considered to be the most important sink for pharmaceuticals to the aquatic environment. Little is known up to now how pharmaceuticals residues behave on passing through the wastewater in the treatment plant and the processes through which they are eliminated from the wastewater. The process of elimination of pharmaceuticals and personal-care product residues within wastewater treatment has to be explored fully to understand ways to reduce concentrations of pharmaceuticals in full scale wastewater treatment effluent. Elimination processes of micropollutants depend on wastewater properties (such as chemical oxygen demand, pH, temperature), the kind of stage treatment process (primary, secondary, tertiary treatment), the kind of biological treatment (activated sludge, oxidation ditch, rotating biological contactor or trickling filter) and the chemico-physical properties of the substance.

This study investigates the fate and removal of Triclosan and Tetracycline in full scale wastewater treatment works and aims to find a correlation between

- The process type of wastewater treatment
- The characterisation of biomass *and*
- The Potential for removal of the selected pharmaceuticals

Four WTPs were selected with different biological treatment processes as representatives for different wastewater treatment. Those different biological treatment steps were

- Rotating Biological Contactor (RBC)
- Trickling Filter (TF)
- Oxidation Ditch (OD)
- Activated Sludge Plant (AS).

The rotating biological contactor and the trickling filter plant were chosen as representative of fixed bed films and the oxidation ditch and activated sludge plant as representative of suspended solid biological treatment.

Spot and composite samples were taken each possible treatment step and analysed for the presence of the pharmaceuticals in the liquid phase to give the ability to examine and compare the influence of those different ways of wastewater treatment steps on the elimination of the monitored compounds. Further selective characteristics of liquid phase and, in particular, of biomass were determined to investigate the possibility of any correlation between the presence and removal of the two pharmaceuticals and the make-up of the biomass.

Those selected liquid phase and biomass characteristics included

- Chemical Oxygen Demand (COD)
- Total organic carbon (TOC^*)
- Soluble polymeric substances (SMP as soluble Proteins/Carbohydrates)
- Extracellular polymeric substances of the biomass (EPS as Protein/Carbohydrates)
- Total Solids and Volatile Total Solids
- Suspended Solids/Volatile Suspended Solids
- Particle size distribution and specific surface
- Lipid content of sludge

And as it is also of interest how well antimicrobials might sorb onto different type of biomass and if there might be any hint in comparison of the biomass characteristics and process type

- Preliminary extraction trials for sewage sludge were undertaken *and*
- Extraction of extracellular polymeric substances were examined for their content of pharmaceuticals

All results are summarised in a full scale sampling list and are compared and discussed with literature data.

4 Material & Methods

4.1 General

The aim of this project was to sample different wastewater treatment plants and to examine the samples for their Triclosan and Tetracycline content in the liquid phase. The samples were analysed as well for their bulk and biomass characteristics to obtain comparative data between the different wastewater treatment systems and their different treatment steps and the removal of the detectable pharmaceuticals. The following sections give detailed information about the sampled sites, the sampling techniques, dates, as well as about the analytical methods.

4.2 Full Scale Sampling

Four UK treatment plants were sampled for the determination of Triclosan (Sites A-D) and a fourth, bigger site (D) was sampled for Tetracycline. Site A-C had been sampled previously by Kanda *et al.* (2003). The results of this earlier work will be included in the discussion.

4.2.1 Site A

This work consisted of two RBCs (rotating biological contactor) followed by a reed bed. The works serves a small village and surrounding rural area, which contained no known industrial effluent. The obtainable data for these site were an average flow of 120 m^3d^{-1}, a Population Equivalent (PE) of 405 and a design population of 500 PE. The total surface area of the disks was 9248.6 m^2.

Samples were obtained from the crude inlet (Influent, A1), the settled sewage tank entering RBC 2 (RBC-Influent, A2), the effluent from the RBCs (RBC-Effluent, A3) and the final effluent (Final Effluent, A4). A flow diagram is shown in Figure 4.1. Sludge samples were obtained from the middle disk of the RBC 2 (A5).

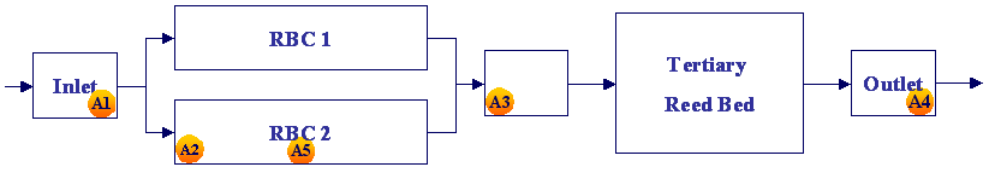


Figure 4.1: Flow chart WTP A - sampling points marked A1 to A5

4.2.2 Site B

Site B treats the wastewater of four catchments, mainly domestic with a little light industry. The trade effluent is 2% of BOD load from light engineering. It was not possible to further specify the type of industry. Treatment consisted of screens and grit removal, followed by two oxidation ditches and final settlement tanks. In total, the site serves an PE of 13,440 and has an average flow rate of 2,700 m^3d^{-1}.

The design water flow (DWF) is 2,881 m^3d^{-1} and the maximum flow treated is 7,145 m^3d^{-1}. The design BOD load is 887 kgd^{-1} and the actual BOD load is 459 kgd^{-1}. The total aeration volume of the two oxidation ditches is 4,320 m^3, nominal hydraulic retention time (HRT) at mean flow 30 hours. The two final settlement tanks each have 12.5 m diameter.

A flow diagram is given in Figure 4.2. with the spot marks for the sampling points. The sampling points at this site were Inlet (Influent, B1), the centre of the Oxidation Ditch (B2) and the Final Effluent (B3).

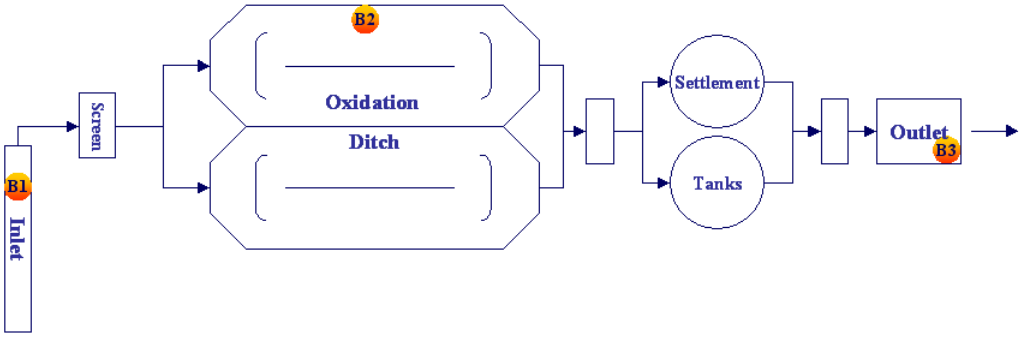


Figure 4.2: Flow chart of WTP B - sampling points marked B1 to B3

4.2.3 Site C

Site C is a standard rural WTP consisting of a settling tank, followed by two trickling filters and two humus settling tanks and finally a polishing lagoon. The incoming sewage

was entirely from domestic catchments with a PE of 2,307 and a consent flow of 1,002 m^3d^{-1}. The design population is 2,750 PE.

The primary settlement has a total volume of 188 m^3, the total surface area is 72 m^2, the surface loading 37.08 m^3d^{-1} The retention time is 1.69 hours. The filter volume of the two trickling filters is 2,256 m^3. The specific surface area of media: 100 m^2/m^3. The filters design loading is 0.12 $kgBODm^3d^{-1}$. The total area of the humus tanks is 56.52 m^2, the total volume 162.96 m^3. The flow to the tanks is 19.41 $Ls^{-1}$and the retention time 1.5 hours.

Samples were taken from the crude inlet (Influent, C1), the settled sewage (Settler, C2), the humus effluent tank (Humus Effluent, C3) and the final effluent (Final Effluent, C4). Flow chart and sampling points are given in Figure 4.3.

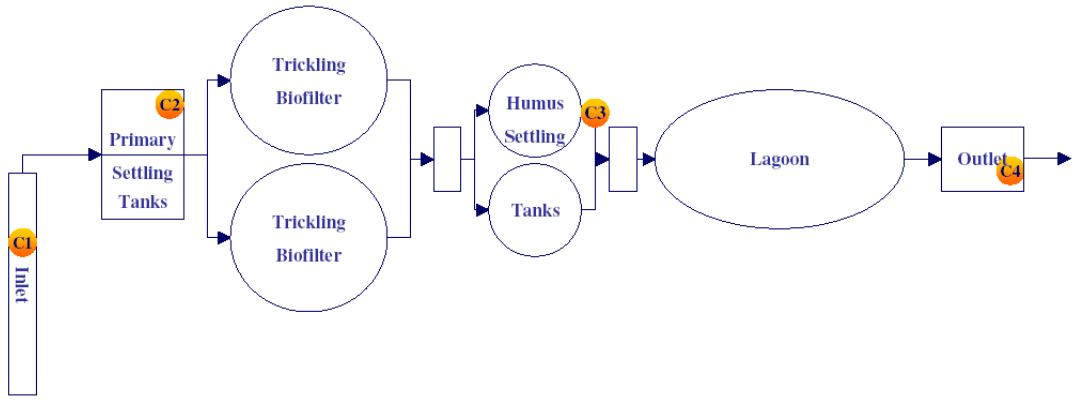


Figure 4.3: Flow chart of WTP C - sampling points marked C1 to C4

4.2.4 Site D

This WTP is the biggest of all four selected WTPs and was chosen for determination of Tetracycline. The site serves two big catchments and consists of 3 activated sludge plants with aerob and anoxic treatment and chemical phosphorus removal. 30% of the BOD load derives from trade effluents from engineering and food waste.

The population served has a PE of 494,387. The design DWF is 100,000 m^3d^{-1}. The actual mean flow is 125,000 m^3d^{-1}, the maximum flow treated 230,000 m^2d^{-1}. The design BOD load is 14,000 kgd^{-1}, the actual BOD load 11,875 kgd^{-1}.

Three Activated Sludge Plant (ASP) modules consist each of 4 aeration lanes (AEROB) and have a total volume of 55,611 m^3 with nominal HRT at mean flow of 11.8 hours and 12 Anoxic zones (one per lane, ANOX) with total volume of 5,223 m^3. Iron is added automatically at the inflow of the ASP. The amount of daily dosage could not be figured out. In addition there are 12 Final settlement tanks each with a diameter of 30.5 m.

Sampling points at this site were the two different inflows (Influent 1, D1 and Influent 2, D2), the outflow of the primary settlement (Settler, D3), the beginning of the anoxic zone (ANOX, D4) and the end of the aeration lane (AEROB, D5), the inflow of the returned activated sludge (RAS, D6) and the outflow of the final settlement (Final Effluent, D7).

Flow chart and sampling points are given in Figure 4.4.

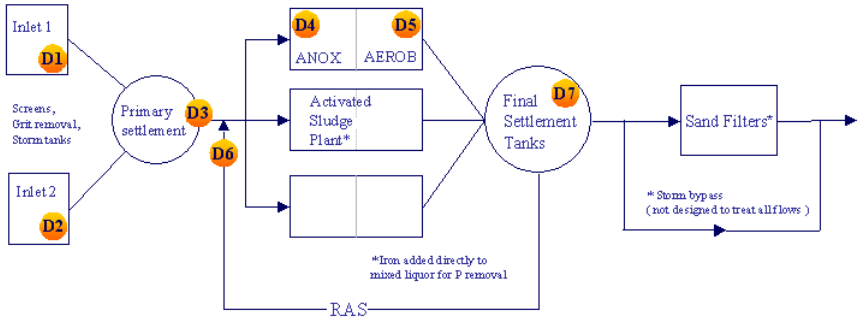


Figure 4.4: Flow chart of WTP D - sampling points marked D1 to D7

4.2.5 Sampling Methods

All sampling points of all four sites were given in the sections above. Sampling points were chosen to obtain a screening of the most important treatment steps at each site regarding the restricted access. No composite sampling were possible till the begin of September and samples had to be taken within the Lone worker-program of Severn Trent, which did not allow the necessary time to sample with hydraulic flow of the sites. Therefore all samples taken by the 9th of September represent grab samples of each step.

Thanks to provision of three automatic sampler from Severn Trent composite sampling was taken for Site B (Oxidation Ditch). A 24 hour composite sampling was analysed for the 9thth of September. An hourly monitoring sampling was carried out from the 13th of September, 10am until the 16th of September, 2am. From the latter every second even hour sample has been analysed for bulk & biomass characteristics for a period of 48 hours, beginning the 13th of September, 10am, ending the 15th of September, 10am. An overview of all sampling data is given in Table 4.1.

Table 4.1: Sampling dates

Date	**Sites Sampled**	**Kind of Sampling**
25th of March 2004	Site A, B, C	grab samples
30th of April 2004	Site A, B, C, D	grab samples
21st of May 2004	Site A, B, C	grab samples
11th of June 2004	Site A, B, C, D	grab samples
01st of July 2004	Site A, B, C, D	grab samples
15th of July 2004	Site A, B, C	grab samples
30th of July 2004	Site D	grab samples
09th of September 2004	Site B	24hour composite sampling
13th of September 2004 10:00 am till 16th of September 2004 10:00 am	Site B	24hour screening for every even hour for a period of 2 days

On site sampling was conducted in accordance with ISO 5667- Part 1,2,3 and ISO 5667-13. Samples were collected in 1L wide neck amber glass bottles which had been pre-cleaned thoroughly and rinsed with acetone and in case for Tetracycline determination with an acetone-EDTA solution. Samples were transported on ice to the laboratory, where some samples were acidified with concentrated sulfuric acid and stored below 4°C till analyse, while other samples were analysed on the day of purchase. A sampling stratification protocol according to conducted analyses is given in Appendix 4.2.5.

4.3 Analytical Work

4.3.1 Chemicals and Materials

Table 4.2: Examined Pharmaceutical Compounds

Name of chemical	Supplier, product number	CAS-Nr.	Formula	Analyti grade [%]	Mr [gMole^{-1}]
Tetracycline hydrochloride	Sigma Aldrich, UK, 250163	64-75-5	$C_{22}H_{24}N_2O_8$.HCl	99.0	480.9
Oxytetracycline hydrochloride	Sigma Aldrich, UK, 46598	2058-46-0	$C_{22}H_{24}N_2O_9$.HCl	95.0	496.9
4-Epi-Tetracycline hydrochloride	Sigma Aldrich, UK, 37918	23313-80-6	$C_{22}H_{24}N_2O_8$.HCl	97.9	480.9
Anhydrotetracycline hydrochloride	Sigma Aldrich, UK, 37919	13-803-65-1	$C_{22}H_{22}N_2O_7$.HCl	98.5	462.88
4-Epi-Anhydrotetracycline hydrochloride	Sigma Aldrich, UK, 37921	4465-65-0	$C_{22}H_{22}N_2O_7$.HCl	97.4	462.88
Triclosan (Irgasan DP300)	Sigma Aldrich, UK, 72779	3380-34-5	$C_{12}H_7Cl_3O_2$	97.0	289.54

Table 4.3: Expendable Items

Name	Supplier	Lot-nr.
OASIS HLB Extraction Cartridges (3cc, 60mg)	Waters Corporation, USA	WAT058883
StrataTM- X Extraction Cartridges (33u Polymeric Sorbent)	Phenomenex, UK	S300-37
Fisherbrand disposable polystyrene cuvettes, 10mm, 4ml	Fisher Scientific, UK	FB 55143
Fisherbrand disposable cuvettes, 10mm, 1.6ml	Fisher Scientific, UK	FB 551447
glass fiber filter (Schleicher and Schuell, Grade GF 52)	Patterson Scientific, Bedfordshire, UK	-
COD test vials, Merck	VWR Internationl Ltd., Dorset, UK	-

Table 4.4: Used Chemicals

Name	**Supplier, Product-nr.**	**Formula**
Total Protein Kit, Petersen Modification	Sigma Aldrich, UK, TP0300	-
Lowry Reagent Powder	Sigma Aldrich, UK, L 3540	-
Folin & Ciocalteu's Phenol Reagent	Sigma Aldrich, UK, F 9252	-
EDTA Dinatriumsalt	BDH Analar, 100935V	$C_{10}H_{14}N_2O_8Na_2.2H_2O$
Oxalic Acid Dihydrate	Fisher Scientific, UK, O/0600/53	$(COOH)_2.2H_2O$
Citric Acid Monohydrate	Fisher Scientific, UK, 12491-0250	$C_6H_8O_7$
Acetic Acid Glacial	Fisher Scientific, UK, A/0406/PB15	CH_3COOH
Acetone	BDH Laboratory Supply, UK, 27023BB	$(CH_3)_2COOH$
Glucose std. Solution ($100 mgdL^{-1}$, 5.56 $mmolL^{-1}$)	Sigma Diagnostics, USA, 635-100	$C_6H_{12}O_6$
Sodium Dihydrogen Orthophosphate Dihydrate	Fisher Scientific, UK, S/3720/53	$Na_2H_2PO_4.2H_20$
Magnesium Sulfate Monohydrate	Sigma Aldrich, UK, 13165	$MgSO_4H_2O$
Standard pH Buffer	VWR International Ltd., 662-4003/4/5	-
Vanillin	Fisher Scientific, UK, AC140820000	$C_8H_8O_3$
Phosphoric Acid, 85%	Fisher Scientific, UK, AC201140000	H_3O_4P
Sodium Hydroxide	Fisher Scientific, UK, 12419-0010	HNaO
Sulphuric Acid, 98%	Fisher Scientific, UK, S/9160/PB17	H_2SO_4
Phenol	Fisher Scientific, UK	C_6H_5OH
Edible Oil	Livio, Unilever	-
n-Hexan	Fisher Scientific, UK, H/0406/17	C_6H_{14}
Iso-propanol	Fisher Scientific, UK, P/7507/15	C_3H_8O
Tetrahydrofuran	Fisher Scientific, UK, 34845-1000	C_4H_8O

4.3.2 Pharmaceutical Determination

4.3.2.1 General

Within this project the determination of the pharmaceutical residues was performed by Reverse Phase High Pressure/Performance Liquid Chromatography (HPLC, Shimadzu LC 10AD) coupled with an UV/VIS detector.

4.3.2.2 Method for liquid phase

All HPLC sample preparation and determinations of the two selected compounds were carried out according to the method set up by A.S. Thompson (Thompson *et al.*, 2004). Samples were examined for the parent compounds Triclosan and Tetracycline as well as for three epimerization products of Tetracycline, epi-Tetracycline, anhydrotetracycline, epi-anhydrotetracycline. Additionally, sludge samples were examined for oxytetracycline, another representative of the tetracyclines family. The possible derivatives of Triclosan did not form the subject of analysis.

Samples were prepared according to given conditions of the methods used by A.S. Thompson, while the HPLC analysis was conducted by A.S. Thompson. Detailed information about the methods are provided in the following paragraphs.

4.3.2.2.1 Preparation of samples One litre of sample was pre-filtrated through glass-fibre filter (Schleicher and Schuell, GF 52). The enrichment was carried out using Strata-X cartridges (Phenomenex, UK) and 3mg Oasis HLB *(hydrophilic-lipophilic balance)* cartridges (Waters, UK) for Tetracycline, Triclosan respectively. The cartridges were preconditioned with 1 mL of methanol (HPLC-grade, Fisher Scientific) and 1 mL of ultra-pure water. The samples were drawn through the cartridges at an approximate flow rate of 3 $mLmin^{-1}$. The cartridges were washed with 1 mL 5% methanol, to remove any loosely bound contaminants, and then eluted with 2 mL methanol.

4.3.2.2.2 Tetracycline determination Tetracycline (*Tetracycline hydrochloride*, CAS 64-75-5), 4-epi-Tetracycline (*4-epi-Tetracycline hydrochloride*, CAS 2331-80-6), anhydrotetracycline (*anhydrotetracycline hydrochloride*, CAS 13-803-65-1), 4-epi-anhydrotetracycline (*4-epi-anhydrotetracycline hydrochloride*, CAS 4465-65-0) and oxytetracycline (*oxytetracycline hydrochloride*, CAS 3380-34-5) were purchased from Sigma-Aldrich, U.K.

Stock solutions were prepared with 1 mgL^{-1} concentration of each above mentioned compound in HPLC-grade methanol (Fisher Scientific, Loughborough, UK) and the solutions were stored at -20°C in an aluminium wrapped amber glass bottle for not longer than 4

weeks. Working standards were prepared on the day of need by diluting the stock solutions to the desired concentration.

The HPLC-UV detection was conducted according to an analytic method, which was based on Oka *et al.* (1984). The HPLC Chromatograph was a Shimadzu LC 10AD. The analytical column was a 150 x 4.6mm ID Xterra C18 column (5 μm, Waters, UK). The mobile phase consisted of 64% 0.2M oxalic acid (Fisher Scientific, UK), 18% acetonitrile (HPLC-grade, Fisher Scientific) and 18% methanol (HPLC-grade, Fisher Scientific). Injection was 10 μL and the flow rate was adjusted to 1 $mLmin^{-1}$. Temperature was kept constant at 30°C. UV response was measured at 400 nm wavelength.

Elution times were according to Thompson *et al.* (2004) 2.0 min for 4-epi-Tetracycline, 2.2 min for Tetracycline, 3.9 min for 4-epi-anhydrotetracycline and 4.8 min for anhydrotetracycline. The limit of quantification (LOQ) was given at 100 ngL^{-1} and limit of determination (LOD) at 50 ngL^{-1}. According to Thompson recoveries were 96% ($\pm$2) for Tetracycline. No recovery rates were provided for the epimerization products.

4.3.2.2.3 Triclosan determination Triclosan (Irgasan, CAS 3380-34-5) was purchased from Sigma-Aldrich, UK. Stock solutions were prepared with 1 gL^{-1} in methanol of each above mentioned compound and the solutions were stored at -20°C in an aluminium wrapped amber glass bottle for not longer than 4 weeks. Calibration standards were prepared on the day of need from that stock solution.

The HPLC detection was developed by A.S. Thompson (Thompson *et al.*, 2004). The HPLC Chromatograph was a Shimadzu LC 10AD. The analytical column was a 150 x 4.6mm ID Xterra C18 column (5 μm, Waters, UK). The mobile phase consisted of 55% acetonitrile (HPLC-grade, Fisher Scientific) and 45% ultra-pure water (Milli-Q). Injection was 10 μL and the flow rate was adjusted to 1 $mLmin^{-1}$. Temperature was kept constant at 30°C. UV response was measured at 235 nm wavelength.

Elution time for Triclosan was according to Thompson *et al.* (2004) at 7.2 min. The limit of quantification (LOQ) was given at 30 ngL^{-1} and limit of determination (LOD) at 1 ngL^{-1}. Recoveries were according to Thompson 94%($\pm$1) for Triclosan.

For the composite sampling different mobile phase conditions have been used by Thompson in terms of better separation. The HPLC mobile phase conditions for samples from the 9^{th} of September and from the 13^{th} of September till the 15^{th} of September was 47.5% of acetonitrile and 52.5% of water instead of the given terms in Table 4.5.

The conditions for the HPLC-UV detection (Shimadzu LC 10AD) according to Thompson *et al.* (2004) are summarised in Table 4.5.

Table 4.5: Analytical conditions for HPLC-UV analysis of Tetracycline and Triclosan (Thompson *et al.*, 2004)

	Tetracycline	**Triclosan**
Liquid Chromatograph	Shimadzu LC 10 AD	Shimadzu LC 10 AD
Column	150 x 4.6mm ID Xterra C18 5μm (Waters, UK)	150 x 4.6mm ID Xterra C18 5μm (Waters, UK)
Injection Volume	10μL	10μL
Mobile Phase	64% 0.2M Oxalic Acid 18% Acetonitrile 18% Methanol	55% Acetonitrile 45% Water
Flow rate	1mLmin^{-1}	1mLmin^{-1}
UV detection	400nm	235nm
Temperature	30°C	30°C
Elution times (minutes)	Epi-tetracycline: 2.0 Tetracycline: 2.2 Epi-anhydrotetracycline: 3.9 Anhydrotetracycline: 4.8	Triclosan: 7.2
Limit of quantification	100ngL^{-1}	30ngL^{-1}
Limit of detection	50ngL^{-1}	1ngL^{-1}

4.3.2.3 Method for sludge extraction

There is an amount of information in the literature about sludge or soil extraction methods for pharmaceutical residues. Within this project there was a trial of pre-work for developing an appropriate method for sewage sludge extraction of the two investigated compounds, Triclosan and Tetracycline.

4.3.2.3.1 Sludge spiking In order to run the first tests of sludge extraction, it is necessary to have sludge samples with a high amount of the compounds of interest. Centrifuged biomass samples (fixed film and activated sludge, 10,300g, 10 min) were weighed into a pre-weighted flask and spiked with either one of each compounds, Triclosan, Tetracycline respectively.

Triclosan spiking was done by dissolving the compound in acetone (used amount 10 mg) and adding to the pre-weighed flaks. Tetracycline spiking, respectively, was done by dissolving the compound in 0.01M hydrochloride acid (used amount 1 mg) and adding to the pre-weight flask. The spiked sludge were thoroughly mixed (5 min, Vortex) and the solvent

were evaporated to dryness. The solvent free sludge were immediately frozen and later on lyophilised.

For gaining recovery rates of the extraction method, theoretically compound-free biomass should have been spiked in varying amounts. As this was not possible, the extraction method ought to be done by adding internal standards.

4.3.2.3.2 Extraction trial for Triclosan 1g of freeze dried sludge sample was weighed into a 100 mL flask and filled with 40 mL 3:2 n-hexane-iso-propanol and 100 μL concentrated sulfuric acid was added. Samples were homogenised (Vortex) for 5 min and continuously stirred for 3 hours. The supernatant was decanted and another 40 mL of the hexane-iso-propanol solution (3:2) was added and procedure repeated. The supernatants were combined. Due to the fact that the used solvents n-hexane and isopropanol are non-polar and were therefore not suitable for reverse phase HPLC, the solvent phase was blown down in a gentle nitrogen stream. The dry residue was reconstituted in 100 mL ultra-pure water, 25 μL sulfuric acid was added and the clean up and concentration was carried out according to section *4.3.2.2.1* by SPE-extraction.

4.3.2.3.3 McIlvaine buffer McIlvaine buffer mixed with EDTA was used to prevent Tetracycline chelation with divalent cations such as metals and calcium or magnesium.

Buffer stock solutions were *Solution A)* 21.01 g of citric acid monohydrate was filled up with deionised water to 1000 mL and *Solution B)* 35.6 g of dinatriumhydrogenphosphatdihydrate filled up with deionised water to 1000 mL.

Mixing stock solutions in appropriate measures results in McIllvaine-buffer at different pH values. Within this work studies were conducted using McIlvaine-buffer at $pH = 4$ (38.6 mL of stock solution B filled up to 100 mL with solution A) and at $pH = 7$ (82.4 mL of working solution B filled up to 100 mL with solution A). 1 mg of Na_2EDTA were added to 100 mL working solution.

4.3.3 Bulk and biomass characteristics

The biomass characterisation was conducted to find possible properties which could be useful predicting pharmaceuticals fate within a certain kind of biomass or wastewater treatment. As the aim of this project is to compare the fate of the selected pharmaceuticals within different treatment steps, the following characterisation tests have been made.

- Chemical Oxygen Demand (COD)
- Total organic carbon (TOC*)
- Soluble Proteins/Carbohydrates
- Extracellular Protein/Carbohydrates
- Total Solids and Volatile Total Solids
- Suspended Solids/Volatile Suspended Solids
- Particle size distribution and specific surface
- Lipids

On site measurement included pH and temperature. It was not possible to measure dissolved oxygen. Due to the given time limit of the lone-worker program of the sponsor, the SVI of the activated sludge tanks could not be determined neither. BOD was not determined and Nitrite, Nitrate and Phosphorous could just be analysed for the composite sampling. A more detailed description of each analysis test is given in the next paragraphs.

4.3.3.1 Preparation of Solid Free Fraction

Preparation of wastewater and sludge fractions (supernatant) free of suspended solids were required for the determination of soluble COD, soluble protein, soluble carbohydrates, nitrite, nitrate and TOC. The solid free fractions of sludge samples were prepared by centrifuging samples for 10 min at 5,000 rpm (4583 g) in a Rotana 96 R centrifuge. The supernatant produced was decanted and filtered through a glass fiber filter (Schleicher and Schuell, Grade GF 52) to remove any residual suspended particles. Wastewater samples were just filtrated without centrifuging.

4.3.3.2 Extraction of Extracellular Polymeric Substances

Extracellular polymeric substances (EPS) were extracted based on the heating method of Zhang *et al.* (1999). 200 ml of biomass sample were centrifuged at 5000 rpm (4583 g) for 10 min and the supernatant is decanted. 200 ml DI water was added to the sludge pellet

and the bottle was hand shaken and placed into the oven until the sludge reached 80°C for 10 min. The bottles were centrifuged while still hot at 7500 rpm (10310 g) for 20 min at room temperature. The supernatant is filtrated through a 70 mm Schleicher & Schuell Grade GF 52 glass fibre filter paper (Patterson Scientific, Bedfordshire, UK).

The method is changed slightly in case the biomass can not be measured volumetric because of its extremely high viscosity (such as fixed films). Then 200 ml DI water is added to $3g$ of centrifuged sludge (10 min at 5000 rpm) and the bottle is hand shaken and heated up as described above. The supernatant is also obtained by centrifuging at 7500 rpm for 30 min and filtration through a GF 52 glass fibre filter.

The obtained supernatants are used for determination of Extracellular Protein, Carbohydrate, COD and for some samples of Nitrite, Nitrate, Phosphorous.

4.3.3.3 Soluble and Extracellular Proteins

Protein concentration by Peterson's Modification was determined by well mixing 1 mL sample with 1 mL of Lowry Reagent Solution (Lowry Reagent, Sigma-Aldrich, Gillingham, UK) and allow solutions to stand at room temperature for 20 minutes. With rapid and immediate mixing, 0.5 mL of the Folin & Ciocalteu's Phenol Reagent Working Solution to each tube. Colour was allowed to developed for 30 minutes. Samples were transfered into cuvetes and measured against a blanc at 595 nm in a Jenway 6505 UV/VIS Spectrophotometer. Adsorbance reading was completed within 30 minutes and results were calculated from a calibration curve obtained from protein standard bovine serum albumin (Sigma-Aldrich, Gillingham, UK). Extracellular Proteins were normalised for liquid sludges against gSS, gVSS respectively. Semisolid and solid sludges were normalised against gTS, gVTS respectively.

The Folin & Ciocalteu's Phenol Reagent Working Solution was prepared by mixing the Folin & Ciocalteu's Phenol Reagent (Sigma-Aldrich, Gillingham, UK) with ultra-pure water (1:6) and stored in an amber glass bottle. The Lowry Reagent Solution was prepared by adding 40 mL of DI water to a bottle of Lowry Reagent Powder (Sigma-Aldrich, Product Code L3540) and mixed well by inverting. Both solutions were kept at room temperature. The Protein Standard solution was stored in the fridge for not longer than 3 weeks. Where necessary samples were diluted to keep adsorbance below 1.0. *See Appendix 4.3.3.3 - calibration curve for proteins.*

4.3.3.4 Soluble and Extracellular Carbohydrates

Soluble carbohydrate concentrations were determined by the phenol - sulphuric acid method (Dubois *et al.*, 1956). 0.4 mL sample of supernatant was added to 0.4 mL of 5% (w/w)

phenol solution (Sigma - Aldrich, Gillingham UK) and then mixed with 2 mL concentrated sulphuric acid (Fisher Chemicals, Loughborough, UK). Colour was allowed to develop for 10 minutes before transfer to cuvetes and the adsorbance measured against a blank at 480 nm (Jenway 6505 UV/Visible Spectrophotometer). Carbohydrate concentration (mgL^{-1}) is calculated from a calibration curve constructed using a glucose standard. Extracellular Carbohydrates were normalised analogous Extracellular Proteins for liquid sludges against gSS, gVSS respectively. Semisolid and solid sludges were normalised against gTS, gVTS respectively. Where necessary samples were diluted to keep adsorbance below 1.0. *See Appendix 4.3.3.4 - calibration curve for carbohydrates.*

4.3.3.5 Chemical Oxygen Demand

The Chemical oxygen demand was determined using Merck Spectroquant COD cell tests. According to the expected COD value, the following Merck cell tests were used:

Table 4.6: Merck Spectroquant cell tests

type of sample	**Measuring range** [$mgCODgL^{-1}$]	**Merck COD cell test n°**
supernatant	10 - 150	1.14540.0001
supernatant	50 - 500	1.14690.0001
supernatant	25 - 1,500	1.14541.0001
sludge	500 - 10,000	1.14555.0001

4.3.3.5.1 Soluble Chemical Oxygen Demand The soluble chemical oxygen demand (SCOD) was determined using Merck Spectroquant COD cell test (VWR International Ltd, Dorset, UK). According to the required sample volume of the Merck cell test a supernatant solid free sample (section 4.2.3.1 or 4.2.3.2) was added to the cell test vial and heated at 150°C for 2 hours. After cooling to room temperature the COD value (mgL^{-1}) was measured in the Spectroquant Nova 60 Spectrophotometer. Samples dilution was not necessary as the range of test kits was broad enough.

4.3.3.5.2 Total Chemical Oxygen Demand The total chemical oxygen demand (TCOD) was determined using Merck Spectroquant COD cell test 1.14555.0001 (500-10,000 mgL^{-1}) (VWR International Ltd, Dorset, UK), analogous to standard method 5220 D APHA (1998). A well mixed homogeneous sludge sample was transferred to the COD test vial. The vial was heated at 150°C for 2 hours and following cooling the total COD value (mgL^{-1}) was measured in a Spectroquant Nova 60 Spectrophotometer (VWR International Ltd, Dorset, UK).

4.3.3.6 Total Organic Carbon

Total organic carbon was measured using a Shimadzu TOC-5000A analyser (Shimadzu, Milton Keynes, UK) with an adapted method for clean water. TOC was calculated by measuring the total carbon (TC) and the inorganic carbon (IC) and subtracting the IC from the TC.

4.3.3.7 Nitrite, Nitrate, Phosphorous, Sulfate

Nitrite, Nitrate, Phosphorous, Sulfate were measured using a Dionex Ion Chromatograph with an adapted method for clean water. The Column was an Ion-Pac AS9-HC. The eluent, 9.0 mM sodium carbonate and the flow rate of 1.0 ml per min. Detection was with suppressed conductivity with an ATLAS suppresser operated at 58 mA .

4.3.3.8 Suspended Solids

Suspended Solids (SS) content was determined by Standard Method 2540D (APHA, 1998). Schleicher & Schuell Grade GF 52 glass fibre filter papers (Patterson Scientific, Bedfordshire, UK) were ignited in a furnace at 550°C for 1 hour and then cooled in a desiccator until needed. The pre-ignited filter papers were weighed immediately prior to use. Well mixed samples were filtered under vacuum through the pre-ignited and pre-weighed filter papers. The volume was chosen to yield a dried residue between 2.5 mg ad 200 mg. The filter papers were subsequently dried in an oven at 105°C overnight, cooled in a desiccator and reweighed. The suspended solids content was calculated according to the following equation:

SS in mgL^{-1} :

$$SS\,[mgL^{-1}] \quad = \quad \frac{(m_{105} - m_c) \cdot 1000}{V_s} \tag{4.1}$$

where:
m_{105}= *weight of dried residue + dish in mg*
m_c = *weight of crucible in mg*
V_s= *volume of sample in mL*

4.3.3.9 Total Solids for liquid samples

Total solids (TS) was determined by Standard Method 2540B (APHA, 1998) for liquid samples. Porcelain crucibles were pre-ignited in a furnace at 550°C for 1 hour and cooled

in a desiccator until required. The crucibles were pre-weighed and fluid sludge was pipetted as a well-mixed homogeneous sample into the pre-weighed crucible. The volume of sample was chosen in a volume of 10 to 30 mL to yield a residue between 2.5 mg and 200 mg. The crucible was left over night in a 105°C drying oven and the evaporated samples were transferred to desiccator for cooling and then re-weighed. Total Solids (TS in mg total solids per L) content is determined according to the following equation:

TS for liquid samples:

$$TS\,[mgL^{-1}] = \frac{(m_{105} - m_c) \cdot 1000}{V_s} \tag{4.2}$$

where:
m_{105}= *weight of dried residue + dish in mg*
m_c = *weight of crucible in mg*
V_s *is the volume of sample in mL*

All analysed sludge samples were done in triplicates. The cycle of drying, cooling, desiccating and weighting was repeated until a constant weight was obtained, or until weight change was less then 4% of previous weight or 0.5 mg, whichever was less.

4.3.3.10 Volatile Suspended Solids and Volatile Total Solids (for liquid samples)

Volatile Suspended Solid (VSS) and Volatile Total Solid (for liquid samples, VTS) contents were determined by Standard Method 2540E (APHA, 1998). The residue obtained from Standard Method 2540D (SS), 2540B (TS) respectively, were ignited at 550° C for 2 hours in a furnace and then cooled in a desiccator upon removal. The dish (crucible or filter paper) was re-weighed and the volatile solids content calculated according to equation below.

Volatile Solids for liquid samples:

$$VSS\,or\,VTS\,[mgL^{-1}] = \frac{(m_{105} - m_{505}) \cdot 1000}{V_s} \tag{4.3}$$

where :
m_{105} *is the weight of the dish containing the dried mass, in mg*
m_{505} *is the weight of the dish containing the ignited dry mass, in mg*
V_s *is the volume of sample in mL*

In addition volatile solids were calculated as loss per ignition in percent of dry mass of a sludge according to EN 12879 (2000):

Loss of ignition [percent]:

$$w_V = \left(\frac{m_{105} - m_{505}}{m_{105} - m_d} \right) \cdot 100 \tag{4.4}$$

where:
w_V is the loss of ignition, in percent
m_{105} is the weight of the dish (crucible or filter paper) containing the dried mass, in grams
m_{505} is the weight of the dish containing the ignited dry mass, in grams
m_d is the weight of the empty dish, in grams

The results are rounded the nearest 0.1%.

4.3.3.11 Total Solids and Volatile Total Solids for semisolid or solid samples

Total solids *(= dry residue)* for semisolid and solid samples, such as fixed biofilms, were determined by EN 12880 (2000).

Porcelain crucibles were pre-ignited in a furnace at 550°C for 1 hour and cooled in a desiccator until required. A sufficient amount of well homogenised wet sludge was put into the pre-weighed crucible that the dried sample's weight yielded at least 500 mg. The crucible was left over night in a 105°C drying oven and the evaporated samples were transferred to desiccator for cooling and then re-weighed. Total Solids (TS in g per kg wet sludge) content is determined according to the following equation.

Total solids for semisolid or solid samples:

$$TS\,(g/kg) = w_{dr} = \left(\frac{m_{105} - m_c}{m_0 - m_c} \right) \cdot 1000 \tag{4.5}$$

where:
w_{dr} is the dry residue of the sludge sample, in grams per kilogram
m_0 is the weight of the crucible containing the wet mass, in grams
m_{105} is the weight of the crucible containing the dried mass, in grams
m_{505} is the weight of the crucible containing the ignited dry mass, in grams
m_c is the weight of the empty crucible, in grams

All analysed sludge samples were done in triplicates. The value should be regarded as constant, if the mass obtained after a further one hour of drying did not differ more than 0.5% of the previous value or 2 mg whichever was the greater.

Volatile Total Solid were analysed analogues 4.4.3.10 and calculated according equation 4.4 (EN 12879, 2000).

4.3.3.12 Particle Size and particle surface

Sludge particle sizes were measured using the Malvern Mastersizer 2000 particle analyser (Malvern Instruments Ltd, Worcestershire, UK). The Mastersizer uses an optical unit to detect the light scattering pattern of sludge particles dispersed in deionised water. Diluted sludge suspensions are circulated through a measurement cell where the particle fields are exposed to an analysing laser beam. The pattern of light scatter can be used to calculate the particle sizes that created the scatter by using Mie theory, which predicts the way light is absorbed and scattered by spherical particles.

All sludge samples were analysed using the same standard operating procedure. The stirrer was set at 350 rpm. Sludge samples were added to the deionised water tank supplying the particle suspension to the measurement cell until the laser obscuration (fraction of light lost by scattering and absorption from the analyser beam) was between 10% and 20%. Ten measurement cycles were taken with a 3 s delay between cycles and an average measurement calculated. The measurement time was set at 20 s (at 1000 snaps/s) in order to ensure that particle size distributions of the sludges were adequately represented by allowing coarser particles enough time to flow through the measurement beam.

The Mastersizer measurement is volume based and according to Mie theory, assumes that the particles causing light absorption and scatter are perfect spheres. Consequently, the results are both volume based and expressed in terms of equivalent spheres. The percentage volume of particles is plotted against particle size (μm). The following parameters are reported:

- Mass Median Diameter (D [v, 0.5]): the particle size (μm) at which, 50% of the sample is smaller and 50% is larger
- D [v, 0.1]: the particle size (μm) below which, 10 % of the sample lies
- D [v, 0.9]: the particle size (μm) below which, 90 % of the sample lies
- D [4, 3]: the volume mean diameter (μm)
- D [3, 2]: the surface area mean diameter (sauter mean) (μm)
- SpSA: specific surface area (m^2g^{-1})

4.3.3.13 Lipid determination

4.3.3.13.1 Standard Method according APHA (1998) Lipids of sludges were determined by A.S. Thompson according the *Standard Method 5220E - Extraction Method for sludge samples* (APHA, 1998). This extraction method is based on a Soxhlet extraction of oil and grease from HCl acidified and Magnesium sulfate monohydrate dried sludge.

20 ±0.5g of centrifuged sludge (10,300g, 5 min) were acidified to pH 2.0 or lower with concentrated HCl and 25g $MgSO_4H_2O$ were added and stirred to a smooth paste. After drying out, the solids were grounded into powder in a porcelain mortar. The powder was added to a paper extraction thimble and the thimble was filled with glass wool. The extraction was conducted with 100 mL of solvent (80% n-hexane, 20% MTBE) in a Soxhlet apparatus at a rate of 20 cycles for 4h. The solvent was recovered and the amount of lipids extracted was calculated.

4.3.3.13.2 Method according to Merck, 1974 Another extraction method of grease and fat has been tried for sewage sludge adapting a method according to Merck (1974). This method has been chosen in favour of sludge extraction. There exists no known record so far which show that this method can be used for determining fat content of freeze dried sewage sludge. The determination of total lipids is based on a method were fat is measured photometrically as sulphophosho-vanillin-complex after opening-up with sulfuric acid at a wavelength of 546 nm.

Vanillin-phosphor-acid: 0.6085 g of Vanillin (Fisher Scientific, UK) was weighed with a little amount of water into a 500 mL volumetric flask and 678.3 g phosphoric acid (Fisher Scientific, 85%) was added, stirred over night in darkness and filled up to 500 mL with ultra-pure water.

One aliquot of the HIP-extract (500 μL) was added into a test tube and blown dry in a gentle nitrogen stream, filled up with 1 mL concentrated sulfuric acid. The test tube was closed, the content well mixed and heated on a water bath up to 100°C for 10 min. The sample was allowed to cool down to room temperature and 50 μL of sample were pipetted off in a new test tube, 1 mL of the Vanillin-phosphoric acid was added and well mixed. After 30 min the sample was measured against a blanc at a wavelength of 546 nm. Calibration curve was gained from a solution of oil in HIP (3:2).

4.3.3.14 Temperature and pH

On site measurement included pH and temperature of the samples. Temperature and pH were conducted by a handheld Hanna HI 8424 pH meter (Hanna Instruments, Leighton Buzzard, UK) calibrated prior each sampling period on basis of standard buffers (VWR International Ltd) and pH measurement was conducted according to EN 12176 (Characterisation of sludge - Determination of pH-value).

5 Results

5.1 Tetracycline

5.1.1 Liquid Phase

Tetracycline and its metabolites were not detected in any of the samples from all four selected wastewater treatment plants.

This might be explained due to their chelating nature of Tetracyclines, which are known to sorb strongly to divalent cations, such as iron, calcium and magnesium (Fe^{2+}, Ca^{2+}, Mg^{2+}), resulting in the formation of relatively stable chelats (Halling-Sørensen *et al.*, 2002). Baker and Brown (1966) stated the ability of of tetracycline binding in metal complexes with Nickel (Ni^{2+}) and Cobalt (Co^{2+}). Furthermore, Loke *et al.* (2002) described the ability of TCs to bind to humic acids and proteins.

Wastewater, biofilms and sludge generally contain significant concentrations of humic acids, proteins and divalent cations, such as Fe^{2+}, Ca^{2+} and Mg^{2+}. So, for instance, from a study conducted by the Environmental Federal Agency of Austria, sewage sludge samples from communal wastewater treatment plants contained concentrations of iron from 7.000 up to 80.000 mg kg^{-1} TS, calcium concentrations of up to 99.800 mg kg^{-1} TS and magnesium of up to 18.500 mg kg^{-1} TS (Umweltbundesamt Wien, 1995).

Therefore, it is more likely that, if any tetracycline(s) were present within the wastewater samples, they are to be found bound to solid samples, such as sludge samples. This is in agreement with the results from other studies which have shown that tetracyclines can sorb strongly to soil organic matter and mineral particles and are therefore rarely found in the free form in surface waters (Christian *et al.*, 2003; Tolls, 2001; Hirsch *et al.*, 1999; Samuelsen *et al.*, 1992; Daughton and Ternes, 1999; Hamscher *et al.*, 2002).

Nevertheless, looking at prescribed doses of tetracyclines in Germany in 1995 *(see Table 2.4 - Excretion rate of tetracyclines and metabolites (Hirsch* et al.*, 1999))* it might also be suggested that tetracycline, , is not likely to be used in relevant quantities for human medical care. Within the class of tetracyclines antibiotics the representatives of doxytetracycline, minocycline and chlortetracycline are those which are currently administered nowadays in high quantities for human medical care (Hirsch *et al.*, 1999).

According to analysis of hospital wastewater effluents undertaken by Färber and Skutlarek (2004) concentrations of doxy- and oxytetracycline were found in concentrations up to 186 ng L^{-1}, whereas concentrations of tetracycline were below the LOQ (*see Table 2.6*).

Boxall *et al.* (2003) predicted a high risk for tetracycline, oxytetracycline, chlortetracycline to enter the environment due to excess use in herd and agriculture treatment. Assuming herd use was the only significant source for tetracycline, it could therefore only enter the sewer systems in measurable quantities due to discharges from, for instance, confined animal farms, or due to run-offs and infiltrations to sewer systems. This was, however, not likely to occur within the sampled catchments.

5.1.2 Sludge Extraction

Preliminary trials for the determination of tetracycline in biofilms and sewage sludge were undertaken in order to determine the extent of PPCPs sorption onto solids such as sewage sludge, after passage thorugh wastewater treatment plant.

Extraction tests were set up according to a modified method used by Sczesny *et al.* (2003) with spiked samples from the oxidation ditch (Site B) and non-spiked samples from the activated sludge plant (Site D). Samples from the oxidation ditch were spiked at concentrations ranging of 5, 10, 20, 50, 100 μg g^{-1} dm of tetracycline hydrochloride and tetracycline hydrochloride combined with 4-epi-tetracycline hydrochloride, anhydrotetracycline hydrochloride, 4-epi-anhydrotetracycline hydrochloride, and oxytetracycline hydrochloride. Samples were lyophilised, extracted according to Sczesny *et al.* (2003) and the residues after evaporation to dryness were reconstituted according the method used by Thompson *et al.* (2004).

As determination could only be undertaken by HPLC-UV detection, chromatograms resulted in very close, partly overlapping and not definable peaks. This may be well a result of interfering compounds within the high organic contaminated sewage sludges and highlights to the need for further examination regarding better clean-up processes in the sample preparation. It might also be caused as well by the structure of tetracyclines. Tetracycline antibiotics exhibit wide variation, however members of the group class reassemble each other due to their common origin (Onken, 1985). Their instability might lead to a situation in which small amounts of structurally related compounds and by-products may be present together. Therefore, it might be very difficult to determine a small amount of degradation product in a vast excess of parent drugs and a high interference of matrix effects (Samuelsen *et al.*, 1992), especially when using only HPLC-UV.

5.2 Triclosan

5.2.1 Liquid phase

Triclosan was detected in almost all of the samples in vast array of concentrations. This was expected, as Triclosan is a widely used *'down-the-drain'*-product (household products which usually enter sewer systems through drains), and has been found in high concentrations during several studies of influent and effluent samples from conventional wastewater treatment plants (Kolpin *et al.*, 2002; Singer *et al.*, 2002; Bester, 2003; Mezcua *et al.*, 2004; Sabaliunas *et al.*, 2003; Lindström *et al.*, 2002). The following sections give further details on each sampling site and data will be compared and, correlated with the obtained data from the biomass characteristics.

The data of different sites will be given in alphabetical order, except from Site B, which will be analysed and discussed as last, as there are lot more data for both conducted studies of grab sampling and 48-hour monitoring composite sampling period.

As mentioned before, Triclosan data obtained by Kanda *et al.* (2003) *(two data point for Dec 01)*, and one sampling period conducted by A.S. Thompson *(Nov 03)* will be included in the Triclosan graphs to give an optimum basis for possible seasonal changes comparison and also to show if there might be any significant differences in the analytical work. Triclosan results will be analysed in *Section 5.3*, whereas biomass characteristics will be given separately in *Section 5.4*.

5.2.1.1 Site A (Rotating Biological Contactor)

Site A has been chosen as a case study since its biological treatment consists of Rotating Biological Contactor (RBC) and final clarifying is done by reed bed. As mentioned previously, grab samples were taken from the crude influent (Influent, A1), the influent to one of the two RBCs (RBC Influent - A2), the effluent of the same RBC (RBC effluent - A3) and the final effluent after the reed bed (Final Effluent - A4). Sludge samples were obtained from the middle row of the sampled RBC (sludge - A5).

Figure 5.1 shows all obtained Triclosan data with the adjusted overall removal of each sampling period. The *adjusted* overall removal represents either the Influent (A1) - Final Effluent (A4) overall removal or the Influent (A1) - RBC-Effluent (A3) overall removal, whichever was higher, obtainable respectively. This depiction has been chosen, as the grab samples do not represent any hydraulic correlation, and therefore the *'real'* overall removal could be significantly different, especially when considering the assumed long retention time within the reed beds (*estimated retention time 2 days - P.Griffin*). All Triclosan data are provided in Table 5.1.

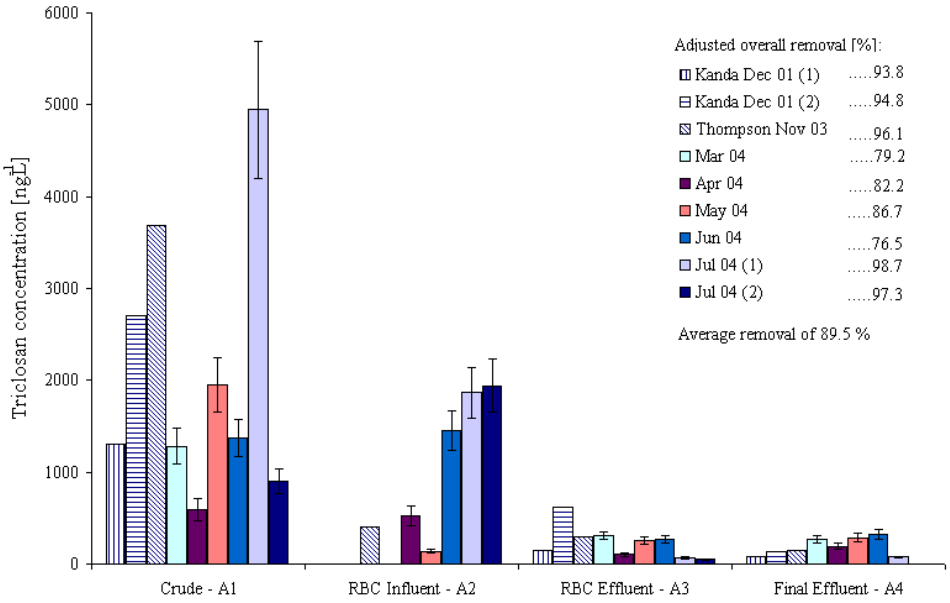


Figure 5.1: Triclosan concentration - Site A (RBC and reed bed)

Table 5.1: Triclosan concentrations - Site A

Triclosan concentrations [ngL^{-1}] - Site A (Rotating Biological Contactor)					
Date	Influent (A1)	RBC Influent (A2)	RBC Effluent (A3)	Final Effluent (A4)	Adjusted overall removal [%]
Kanda Dec 01(1)	1300	no data	150	81	93.8
Kanda Dec 01(2)	2700	no data	620	140	94.8
Thompson Nov 03	3687	404	302	145	96.1
Mar 04 *(25-Mar-04)*	1282	no data	310	267	79.2
Apr 04 *(30-Apr-04)*	594	526	106	194	82.2
May 04 *(21-May-04)*	1948	136	256	289	86.9
Jun 04 *(11-Jun-04)*	1371	1455	272	322	76.5
Jul 04(1) *(01-Jul-04)*	4945	1865	66	75	98.7
Jul 04(2) *(15-Jul-04)*	897	1939	52	n.d.	97.3
average	2080	1054	237	189	89.5

n.d. - not detectable

The adjusted overall removal for Site A is between 76.5% *(Jun 04)* and 98.7% *(Jul 04(1))*. The median for all conducted studies is 89.5%, with concentrations ranging from 594 ngL^{-1} to 4,945 ngL^{-1} and 75 ngL^{-1} to 322 ngL^{-1} for influent, final effluent respectively. The median inflow concentrations are 2,080 ngL^{-1} and 189 ngL^{-1} for the effluent.

Interestingly, the highest influent concentration of 4,945 ngL^{-1} is for the sampling of the 1st of July (*Jul 04(1)*), where highest overall removal is also achieved. This might be an indicator for well adapted microorganisms and high removal rates in warmer seasons, but cannot be concluded certainly because of the fact that the samples do not correlate to the hydraulic flow. In fact, with reference to the the values of Kanda *et al.* (2003) and A.S. Thompson for the months December 2001 and November 2004 *(Dec 01(1,2), Nov 03)* into account, no significant changes in the overall removal were observed.

Considering the removal rates between crude influent (A1) and RBC influent (A2) for slightly comparable data (*Nov 03, May 04, Jul 04(1)*), the conclusion might be reached that a significant loss of Triclosan is due to settling processes, regarding the receiving tank of the inflow of the RBC as a small settling tank.

Another significant removal seemed to occur in the biological stage of this wastewater treatment plant. The removal of Triclosan between the RBC influent and the RBC effluent resulted, for instance, in the sampling conducted in July (*Jul 04(2)*), in an observed elimination of 97.3%. Examining the data value of November 2003 *(Thompson Nov 03),* where no significant loss can be observed after the biological treatment step, and the data value of December 2001 *(Kanda Dec 01(1))*, where the value after the biological treatment step, are up to twice as high as all other samples, the disappearing rate of Triclosan might well be influenced by temperature changes and therefore reduced biological activity within the biomass.

No significant influence on the the overall removal of Triclosan can be observed due to the reed bed. Except for the data of Kanda *et al.* (2003) *(Dec 01 (1,2))* and Thompson *(Nov 03)* no relevant losses seemed to occur between the effluent of the RBC and the final effluent. On the other hand, this might also be a result of the fact of the non-flow correlating sampling.

Assuming a constant daily concentration for Triclosan and a constant average flow of the site, the daily load of Triclosan would be 0.22 g per day for the influent and 0.0275 g per day for the effluent. This would result in a per capita output of 545 μg per day for the influent, assuming no losses in the sewer systems, and 68 μg per capita and day output with the WTP discharges. *(Calculation tables can be found in Appendix 5.2.1)*

5.2.1.2 Site C (Trickling Filter)

Site C was chosen as it included a biological trickling filter and represents, in addition to Site A (RBC), another examples of fixed biofilms. Samples were taken from the crude influent (Influent - C1), the settling tank (Settler - C2), the effluent of the humus settling tank (Humus Effluent - C3) and the final effluent (Final Effluent - C4). Unfortunately, no biomass samples could be taken from the trickling filters.

Figure 5.2 shows all obtained Triclosan data with the adjusted overall removal of each sampling period. Any calculation tables can be found in *Appendix 5.2.2.* The overall removal is again expressed as adjusted overall removal due to the fact that no final effluent data were available for Kanda *et al.* (2003). In addition, on one occasion the humus effluent concentration was slightly higher than the measured final effluent content, which might well be due to the non-hydraulic flow correlated samples.

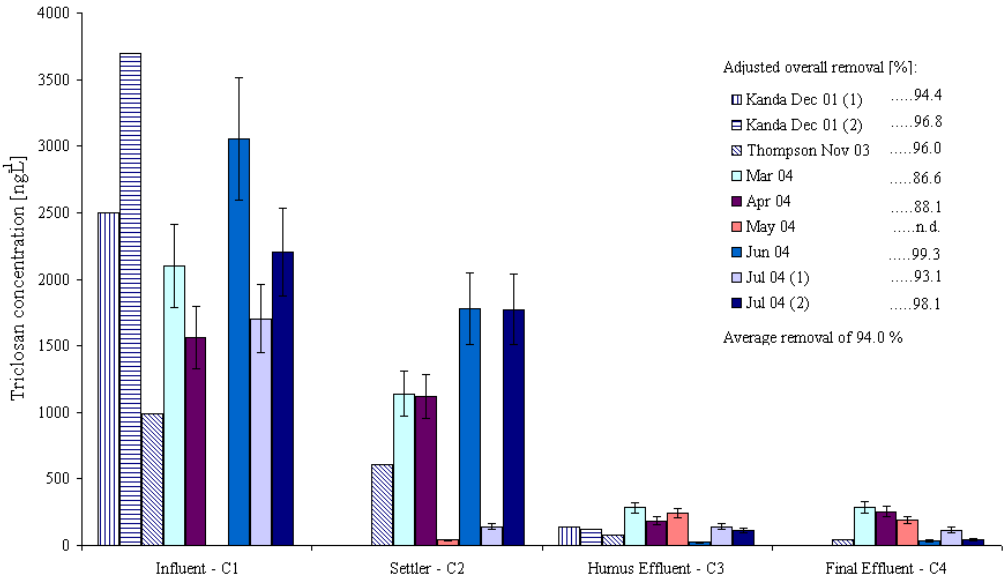


Figure 5.2: Triclosan concentration - Site C (Trickling filter)

The adjusted overall removal of Site C is between 88.1% *(Apr 04)* and 99.3% *(Jun 04).* The median for all conducted studies is 94.0%, with concentrations ranging from 993 ngL^{-1} *(Nov 03)* to 3,700 ngL^{-1} *(Dec 01(1))* and 35 ngL^{-1} *(Jun 04)* to 290 ngL^{-1} *(Mar 04)* for influent, final effluent respectively. The median inflow concentrations are 2,227 $ngL^{-1}$and 138 $ngL^{-1}$for the effluent.

Table 5.2: Triclosan concentrations - Site C (Trickling Filter)

Triclosan concentrations [ngL^{-1}] - Site C (Trickling Filter)					
Date	Influent (C1)	Settler (C2)	Humus Effluent (C3)	Final Effluent (C4)	Adjusted overall removal [%]
Kanda Dec-01(1)	2500	no data	140	no data	94.4
Kanda Dec 01(2)	3700	no data	120	no data	96.8
Thompson Nov-03	993	609	75	40	95.7
Mar 04 *(25-Mar-04)*	2100	1141	283	290	86.5
Apr 04 *(30-Apr-04)*	1562	1118	186	254	88.1
May 04 *(21-May-04)*	no data	40	244	191	-
Jun 04 *(11-Jun-04)*	3057	1779	(22) <LOQ	35	99.3
Jul 04(1) *(01-Jul-04)*	1703	141	141	117	93.1
Jul 04(1) *(15-Jul-04)*	2202	1773	116	42	98.1
average	2227	943	147	138	94.0

n.d. - not detectable; LOQ - Limit of quantification

According to the overall removal rates found by Kanda *et al.* (2003) *(Dec 01 (1,2))* and Thompson *(Nov 03)*, no significant changes were observed due to varying seasonal conditions. The lowest overall losses seemed to occur, in fact, in the months of March *(Mar 04)* and April *(Apr 04)*, when temperatures are moderate. This might well be caused by the sampling stratification, where samples do not correlate with the hydraulic flow. On the other hand, samples were taken in March and April under cloudy conditions and directly after rainfall periods. As there were no flow data available, it just can be assumed that the rainfall might have had an influence on the hydraulic load of the site and therefore caused a decrease in hydraulic retention time and a subsequent decrease in Triclosans overall removal.

Samples conducted in June *(Jun 04(1))* and July *(Jul 04(1))* showed the highest influent concentrations in this study (besides *Kanda (Dec 01 (2))*) and the apparently highest removal rates of up to 99.3% *(Jun 04(1))*. This might be due to increased biological activity in the biofilter, due to settling processes in the settling tank or even due to phototransformation processes either in the settling tank or humus effluent tank. A more specific statement can not be given according to the sampling points and the fact that no determination methods for degradation products existed for this study.

Assuming a constant daily concentration for Triclosan and average inflow of 1,002 $m^3 day^{-1}$, the daily load of Triclosan would be 2.1 g per day for the influent and 0.16 g per day for the

effluent. This would result in a per capita input of 923 μg per day to the WTP, assuming no losses in the sewer systems, and 67 μg per capita and day output with the WTP discharges.

5.2.1.3 Site D (Activated Sludge Plant)

Site D was chosen as it represents one very important treatment plant for the Sponsor Severn Trent *(PE 494,387)* and because it consists of activated sludge modules with anoxic and aerobic lanes and chemical phosphorous removal. Sampling Site D was originally of interest for tetracycline determination, but none of the parent compound or degradation products could be detected *(see section 5.2)*. Sampling points were the two influents *(D1 and D2)*, the effluent of the primary settling tank *(Settler D3)*, the anoxic zone of the activated sludge plant *(ANOX D4)*, the aerobic zone of the activated sludge plant *(AEROB D5)*, the returned activated sludge *(RAS D6)* and the effluent of the final clarifier *(Final Effluent D7)*. Biomass determinations were carried out using the three sludge samples D4, D5, D6 and results will be given in Section 5.4.2.

Site D was not sampled by Kanda *et al.* (2003) *(Dec 01)* nor by Thompson *(Nov 03)* and sampling was conducted for only four sampling dates *(Apr 04, Jun 04, Jul 04 (1) and Jul 04(2))* depending on safety regulations.

An overview of all Triclosan data is given in Figure 5.3, whereas the specific concentration values are given in Table 5.3.

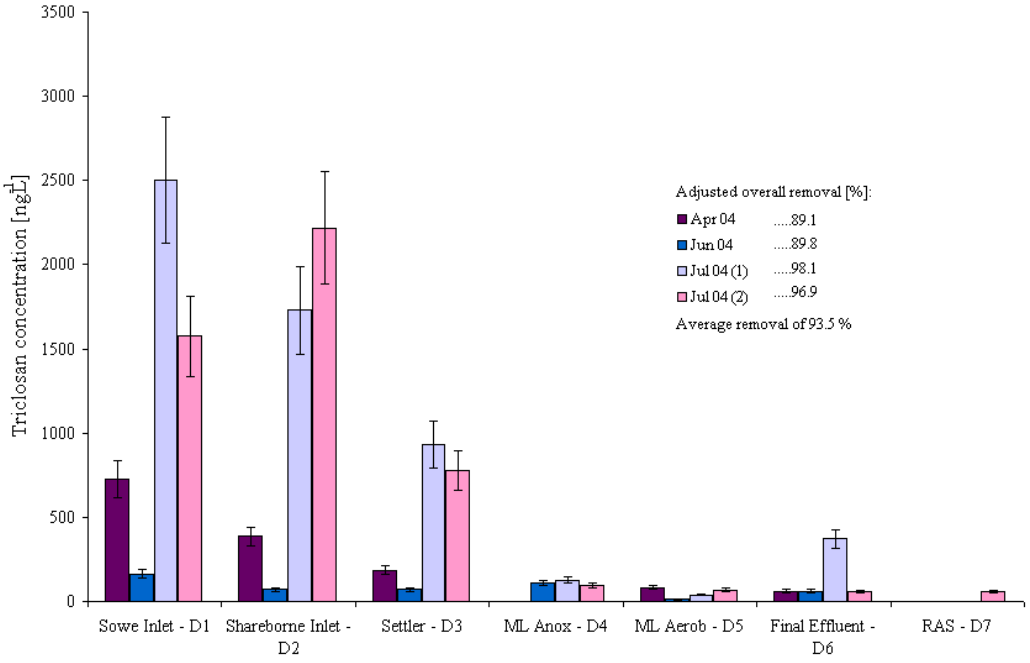


Figure 5.3: Triclosan concentration - Site D (Activated Sludge Plant)

Table 5.3: Triclosan concentrations - Site D (Activated Sludge Plant)

Triclosan concentrations [ngL^{-1}] - Site D (Activated Sludge Plant)

Date	Influent 1 (D1)	Influent 2 (D2)	D1+D2 (1:1)	Settler (D3)	ANOX (D4)	AEROB (D5)	RAS (D6)	Final Effluent (D7)	Adjusted overallremoval [%]
Apr 04 *(30-Apr-04)*	728	386	557	187	n.d.	83	n.d.	61	89.1
Jun 04 *(11-Jun-04)*	164	71	118	73	110	(12) < LOQ	n.d.	61	89.8
Jul 04(1) *(01-Jul-04)*	2504	1729	2117	930	127	40	n.d.	371	98.1
Jul 04(2) *(30-Jul-04)*	1575	2219	1897	776	94	67	59	59	96.9
average	1243	1101	1172	492	110	51	-	138	93.5

n.d. - not detectable; LOQ - Limit of quantification

Neglecting the fact of non-flow correlating samples and assuming a proportional inflow of 1:1 for the influents D1 and D2, Site D also showed a relatively high adjusted average removal of 93.5%. The highest adjusted overall removal was 98.1% *(Jul 04(1))* assuming the aerobic lane concentration as an effluent value for this specific occasion. Otherwise the actual measured overall removal of *Jul 04(1)* would exceed only 85%. However, the sampling conducted in *Jul 04(2)* showed an overall removal of 97%.

The Triclosan concentrations ranged from 74 ngL^{-1} *(D2, Apr 04)* to 2,504 ngL^{-1} *(D1, Jul 04(1)),* and 59 ngL^{-1} *(Jul 04(1))* to 371 ngL^{-1} *(Jul 04(1))* for influent, final effluent respectively. According to inflow ratio of 1:1 for influent 1 *(D1)* and influent 2 *(D2)* the lowest concentration was 118 ngL^{-1} *(Jun 04)* and the highest 2,117 ngL^{-1} *(Jul 01(1)).* The median inflow concentrations are 1,172 ngL^{-1} *(D1+D2)* and 138 ngL^{-1} for the final effluent *(D7).*

Regarding the relative removal of Triclosan within the flow of the wastewater treatment plant, it might be concluded that on average almost 60% of Triclosan are retained within the primary settling process, whereas 40 % seem to be removed in the activated sludge tank. Only a slight decrease of Triclosan concentration can be seen within the final clarifier. This is in accordance with other studies (Kuch *et al.*, 2003; Schneider *et al.*, 2004*b*).

The supernatant of the returned activated sludge *(RAS, D7)* did not show any significant *re-input* of soluble Triclosan. In fact, it was only detectable once with a concentration of 59 ngL^{-1} *(Jul 04(2)).* However, it can not be concluded that almost no Triclosan will be recirculated in average as it might well be sorbed to the biomass. On the other hand, it may also be that Triclosan underwent mostly biodegradation as it has been reported by a number of researchers (McAvoy *et al.*, 2002; Singer *et al.*, 2002; Bester, 2003).

Assuming again a constant Triclosan concentration during the day and a constant average flow of the site, the daily load of Triclosan in WTP D would be 147 g per day for the influent and 8 g per day for the effluent. This would result in a per capita input of 296 μg per day to the WTP and 15 μg per capita and day output with the WTP discharges into the environment. *(Calculation tables can be found in Appendix 5.2.3)*

5.2.1.4 Site B (Oxidation Ditch)

Site B was chosen because it consists of an Oxidation Ditch and is therefore representative of long hydraulic retention times (30 h at mean flow). Sampling points were the crude influent *(B1)*, the oxidation ditch *(B2)* and the final effluent *(B3)*.

In common with all three previous sites Site B was sampled in grab samples *(Mar 04 - Jul 04)* and also in composite samples *(Sep 04)*. This WTP was also included in the study conducted by Kanda *et al.* (2003) *(Dec 01(1,2))* and has been sampled by Thompson *(Nov 03)*. In section 5.2.1.4.1 the results from Kanda *et al.* (2003) *(Dec 01(1,2)*, Thompson *(Nov 03)*, the sampling period *March 04 - July 04* and the mean of the composite sampling *(Sep04)* is analysed, whereas section 5.2.1.4.2 examines the data obtained from the 48 hour monitoring composite sampling.

5.2.1.4.1 Grab samples The obtained results are presented in Figure 5.4 and Table 5.4 present of the sampling period March 2004 - July 2004, the mean from the composite sampling in September 2004 and as well the data from Kanda *et al.* (2003) *(Dec 01)* and Thompson *(Nov 03)*.

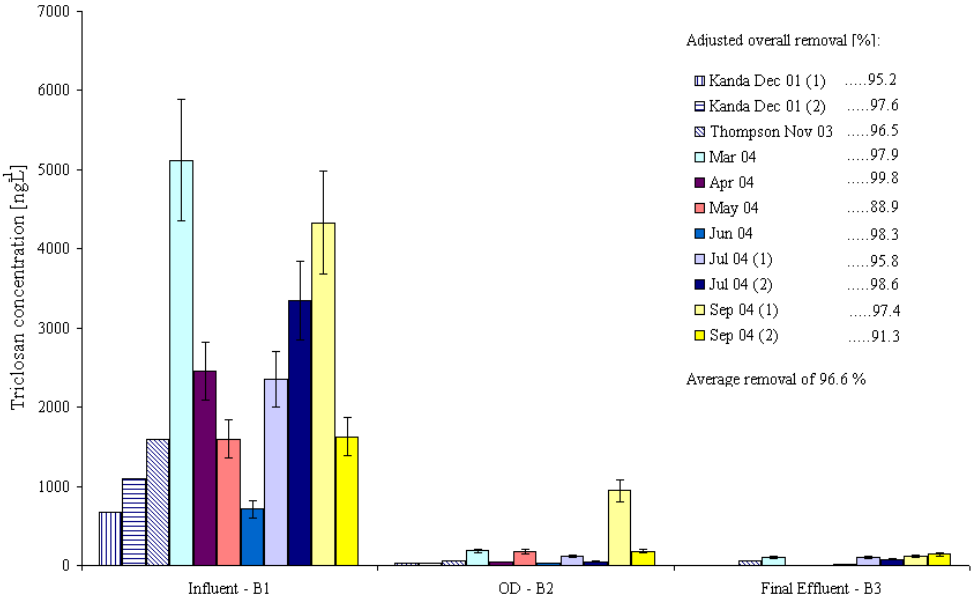


Figure 5.4: Triclosan concentration - Site B (Oxidation Ditch)

Table 5.4: Triclosan concentrations - Site B (Oxidation Ditch)

Triclosan concentrations [ngL^{-1}] - Site B (Oxidation Ditch)				
Date	Influent (B1)	Oxidation Ditch (B2)	Final Effluent (B3)	Adjusted overall removal [%]
Kanda Dec-01(1)	6700	no data	32	95.2
Kanda Dec-01(2)	1100	no data	27	97.5
Thompson-Nov 03	1598	62	56	96.5
Mar 04 *(25-Mar-04)*	5115	183	104	98.0
Apr 04 *(30-Apr-04)*	2451	43	(4) <LOQ	99.8
May 04 *(21-May-04)*	1596	177	n.d.	88.9
Jun 04 *(11-Jun-04)*	710	(29) <LOQ	(12) <LOQ	98.3
Jul 04(1) *(01-Jul-04)*	2349	117	99	95.7
Jul 04(2) *(15-Jul-04)*	3347	47	80	98.6
Sep 04(1) *(09-Sep-04*)*	4328	943	112	97.4
Sep 04(2) *(14-Sep-04**)*	1627	181	141	91.3
average	2263	167	76	96.6

n.d. - not detectable ,*composite sampling, **24h mean (Sep 14th) of the 48h monitoring study

The overall removal is approximately 97%, not taking the sampling data for *May 04* into account. The apparently highest average removal is for *Apr 04* with 99.8%, whereas the lowest average loss seemed to be for *May 04* with 88.9% and *Sep 04(2)* with 91.3% respectively.

The influent concentration ranged from 710 ngL^{-1}*(Jun 04)* to 5,115 ngL^{-1}*(Apr 04)*. The highest effluent concentration was for the composite sampling *Sep 04(2)* with 141 ngL^{-1} and the lowest measured concentration 4 ngL^{-1}, within the limit of quantification for *Nov 03 (Thompson)* 56 ngL^{-1}, respectively. Regarding the high overall removal for the the winter period no significant changes were observed with varying seasons. In fact, the oxidation ditch represents the most consistent biological treatment regarding overall removal rates within this study. This is in accordance with one study conducted by Schneider *et al.* (2004*b*) *(see Table 6.1)*.

Assuming again a constant Triclosan concentration during the day and a constant average flow of 2,700 m^{3}d^{-1}, the daily load of Triclosan in Site B would be 7.0 g per day for the influent and 0.162 g per day for the effluent. This would result in a per capita input of 521 μg per day to the WTP and 12 μg per capita and day output with the WTP discharges.

Table 5.5 gives an overview of the calculated Triclosan input an output during the grab sampling period and the composite sampling and monitoring study.

Table 5.5: Triclosan mass flow - Site B

Site B - Triclosan mass flow results for sampling period March 04 - Oct 04								
Date	Flow rate [m^3d^{-1}]	Triclosan concentration [ngL^{-1}]		Load of Triclosan [$gday^{-1}$]		Triclosan load in μg day^{-1} per capita		Overall removal [%]
		Influent	*Effluent*	*Influent*	*Effluent*	*Influent*	*Effluent*	
Grab sampling period	2,700	2,595	60	7.0	0.162	521	12	97.9
Sep 04(1)	2,514	4,328	112	10.9	0.282	810	21	97.4
Sep 04(2)	3,898	1,627	141	6.3	0.550	427	41	91.3
average		2,690	79	7.4	0.2	551	17	96.8

With the given discharge flow rates of 2,514 m^3d^{-1} for the 9th of September 2004 *(Sep 04 (1))* and 3,898 m^3d^{-1} for the 14th of September 2004 *(Sep 04 (2))*, and assuming an ideal condition being effluent flow rate equal to influent flow rate, the mass flow for the composite sampling was 6.3 g day^{-1} *(Sep 04 (2))* to 10.9 g day^{-1} *(Sep 04 (1))* input and 0.28 day^{-1} *(Sep 04 (1))* to 0.55 g day^{-1} *(Sep 04 (2))* output. This would result in 810 *(Sep 04 (1))*, 337 *(Sep 04 (2))* μg per capita and day mass inflow and 21 *(Sep 04 (1))* - 41 *(Sep 04 (2))* μg per capita and day mass outflow. *(further calculation tables can be found in Appendix 5.2.4-1)* .

The differing mass outflow values of Triclosan could be related to the higher hydraulic load of the WTP. The values for the 09th of September *(Sep 04 (1))* show much higher influent load, but just half of the effluent load than the value for *(Sep 04 (2))* where much higher hydraulic load occurred. This will be explained more detailed in the Discussion chapter 6.1 *(Figure 6.5)*.

5.2.1.4.2 48-hour monitoring composite samples A 48-hour monitoring study was conducted using automatic samplers (ISCO). Samples were taken from the Influent, Oxidation Ditch and Effluent every hour and were analysed for Triclosan. Within this project, every second hour was analysed for Triclosan and as well for liquid and biomass characteristics. As it might be obvious that immense and time intensive work capacity was necessary to analyse all 72 samples of this part of the study, sample preparation for Triclosan determination for influent and effluent samples was conducted by A.S. Thompson, whereas preparation of oxidation ditch samples, as well as EPS fraction preparation, and determination of liquid and biomass characteristics for all samples were part of this thesis work. Furthermore, the

analysis of Triclosan content within the EPS was part of this thesis and was undertaken at the Institute of Hydro Chemistry at Dresden University of Technology.

Figure 5.5 represents the Triclosan values of the monitoring study for influent, oxidation ditch and effluent. Full scale sampling list and calculation tables can be found in *Appendix 5.2.4-2*.

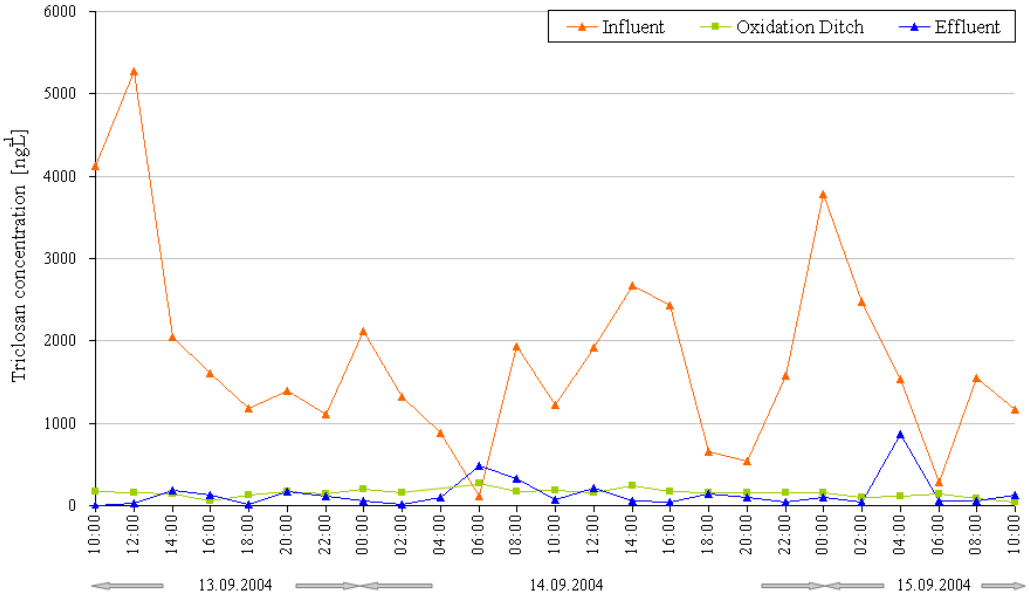


Figure 5.5: Triclosan concentration - Site B - 48 h monitoring composite sampling

The concentration of Triclosan in the influent varied widely over the period monitored from 108 ngL^{-1} to 5,277 ngL^{-1}, with an average of 1,800 ngL^{-1}. The concentration in the oxidation ditch samples ranged from 43 ngL^{-1} to 264 ngL^{-1} and showed much less variability than the influent samples. The effluent samples, however, exceeded the highest value of the oxidation ditch for three sampling points and showed much more variation than the Triclosan results of the oxidation ditch. The highest measured average effluent value was 871 ngL^{-1}, whereas the lowest value was found to be 2 ngL^{-1}($<$ LOQ) and 17 ngL^{-1}.

Since Triclosan is used as an antibacterial agent in common household products such as toothpastes, soaps, and washing up liquids, it is expected to appear within the daily peaks of an WTP, usually occurring in the early morning, around lunch time, and in the early evening. However, the rising mains of the four catchments of Site B end in an intermittently operated pumping station serving the influent of Site B. It was not possible to determine the pumping regime for the sampling period and therefore the daily occurring peaks can

not be directly correlated to consumer patterns. Another fact is that during high rainfall seasons the hydraulic load is diverted into a storm flow bed *(note: not marked in flow chart, Figure 4.2),* where additional desorption of Triclosan residues may occur, which may be pumped back later into the influent. Site B also serves a small amount of light industry, such as laundries, pie factories, resulting on average in 2% of the daily BOD load, which may also result in Triclosan peaks due to their mode of operation.

The oxidation ditch itself proved to be very stable with only slight changes in concentration values. This was as expected because of its long hydraulic retention time and therefore its immense capacity to act as a *'buffer'* for shock loads of contaminants. A final effluent concentration which was much higher than the oxidation ditch concentration was linked during the grab sampling period on the non flow correlated sampling stratification and was not expected at all within this monitoring study. The reasons for this occasional occurrence are, so far, unclear. It might be a result of washed-off Triclosan from the final settling tanks or perhaps of the sampling regime. The pipe of the auto-sampler might have released some biomass from the effluent brickwork, which was taken into sample where desorption occurred, but there is no firm evidence for that. This, in fact, can not be supported by the data of the suspended solids within the effluent samples *(see chapter 5.3).*

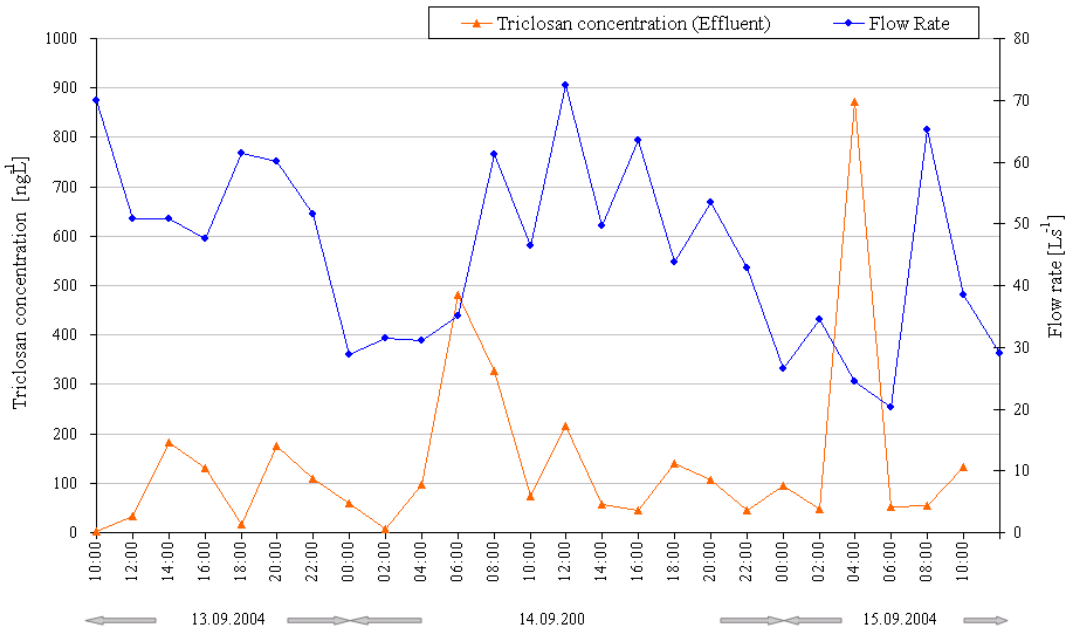
It may also be that desorption occasionally takes place within the final clarifier. In fact, this point will be discussed further in the next section (5.2.3 - *Triclosan content in EPS*).

Figure 5.6 shows the measured Triclosan concentration (ngL^{-1}) and the Triclosan load (content in mgh^{-1}) each related to the effluent flow rate (Ls^{-1}). As it remains unclear what the flow rate was within the wastewater treatment plant until the effluent, these facts remain unclear as well. However, Antusch (1999) stated that sorbed Triclosan in the biomass of sewer systems is easily released within increased hydraulic load. Nevertheless, there might be another explanation for the higher effluent values, as they are compared again in section *5.2.3.*

The overall removal during the monitoring study was around 92%. This is slightly lower than the observed values by Kanda *et al.* (2003) and Thompson *(Nov 2003)* and even lower than the samplings undertaken within this study *(see Table 5.4).* A comparison to a study conducted by Schneider *et al.* (2004*b*) can be found in the discussion part *(Table 6.1).*

The daily load of Triclosan was for the influent 6.3 g, and 0.550 g for the effluent for the measured values of the 14^{th} of September.

(a)



(b)

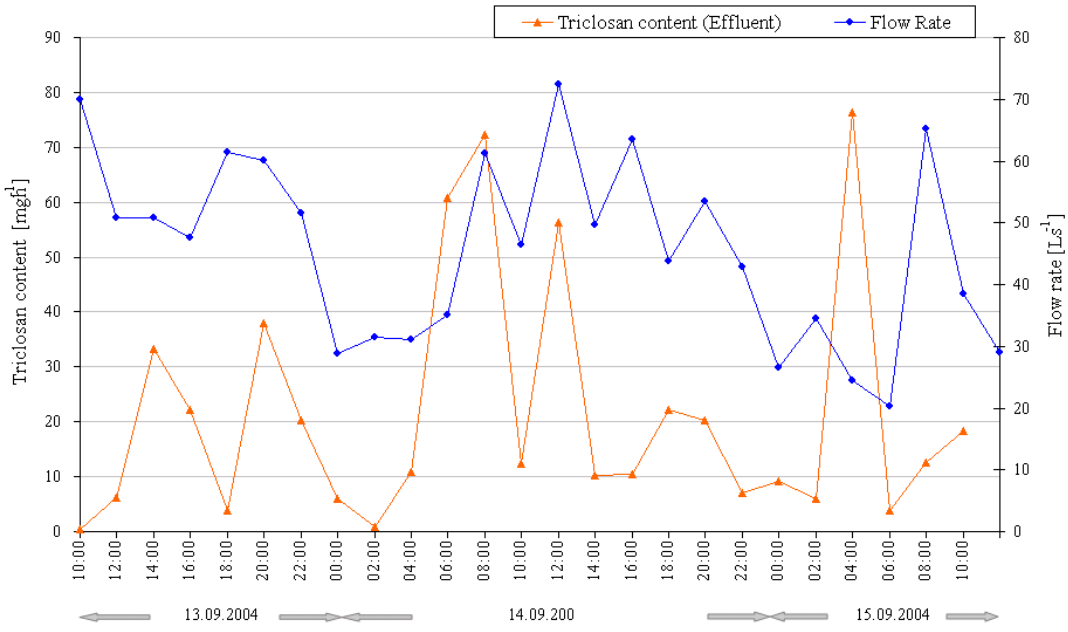


Figure 5.6: Triclosan concentration *(a)* and content *(b)* for effluent, related to flow rate

5.2.2 Sludge Extraction

Of particular interest within this study, was the question relating as to whether the lipid content of biomass sample influences the sorption capacity. Therefore, an extraction method based on Schettgen (2000) was tested, where lipid determination was undertaken according to Merck (1974).

Freeze dried sludge samples have been determined for their Triclosan content as previously described in Chapter 4 *(Material & Methods)*. 1g of freeze dried sludge sample was weighed into a 100 mL flask and filled with 40 mL 3:2 n-hexane-iso-propanol and 100 μL concentrated sulfuric acid was added. Samples were homogenised (Vortex) for 5 min and continuously stirred for 3 hours. The supernatant was decanted and another 40 mL of the hexane-iso-propanol solution (3:2) was added and procedure repeated. The supernatants were combined and the solvent phase was blown down in a gentle nitrogen stream. The dry residue was reconstituted in 100 mL ultra-pure water, 25 μL sulfuric acid was added and the clean up and concentration was carried out according to section *4.3.2.2.1* by SPE-extraction.

Interferences have shown to be very high and therefore no compound could be detected, not even within highly spiked sludge samples (c = *5 mg (kg dm)*$^{-1}$). An additional clean-up step, either before SPE extraction, such as a liquid based clean-up or the use of silica gel to remove the obviously interfering humic substances, or the adding of an additional SPE-clean-up (= *sacrifice cartridge*) might lead to more reliable results. The use of an internal standard, such as pentachlorophenol will also be necessary to determine recovery rates and assist with stabilising the analytical detection. As no additional clean-up equipment was available and optimising the sludge extraction would have been too time intensive for this thesis work, another trial has been undertaken as a substitution for the sludge extraction and will be discussed more detailed in the following section.

5.2.3 Triclosan extraction from EPS fraction

As it has not been possible to determine sludge extracts for Triclosan content, another method was undertaken to investigate how Triclosan sorption could influence the waste-water treatment process. Therefore the oxidation ditch samples from the last sampling stratification (*48-hour monitoring sampling and composite samples of Site B*) have been analysed for their Triclosan content within the extracted EPS fraction.

Samples were divided into 200 mL fractions, centrifuged and the supernatant was used for liquid determination (SMP), whereas the EPS extraction was prepared according to paragraph *4.3.3.2*. The supernatants containing the EPS fraction were recombined and preparation and determination has been undertaken according to section *4.3.2.2*. The

values were recalculated for the extracted amount of suspended solids and were normalised to suspended and volatile suspended solids.

The first extraction trial had been undertaken with the composite sampling of Site B, the 9th September 2004. The EPS extract showed very high amounts for Triclosan compared to the SMP fraction (2:1) of the oxidation ditch. The total concentration found in one litre extracted EPS was 1,991 ngL^{-1}, resulting in 780 ng gSS^{-1}(composite sampling, 9th of September). This high amount combined with the fact that disturbing substances were not as high as for sludge samples, encouraged to carry out the same analyses for the later 48-hour monitoring sampling. The following section presents the results achieved in comparison with the results of the liquid phase and comparison to biomass characteristics are given in Section *5.3*.

Triclosan content

Figure 5.7 shows the results of extracted Triclosan from the extracellular polymeric substances in ng gSS^{-1} and ng gVSS^{-1}. The content of Triclosan varied widely for each sample and showed a maximum of 300 ng per gSS, 400 ng per gVSS respectively.

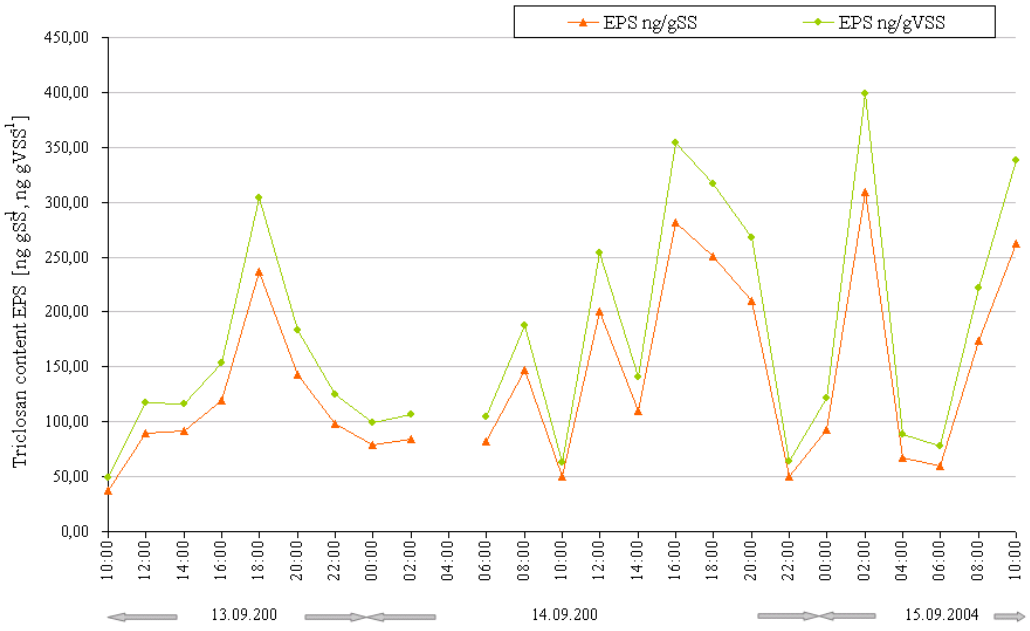
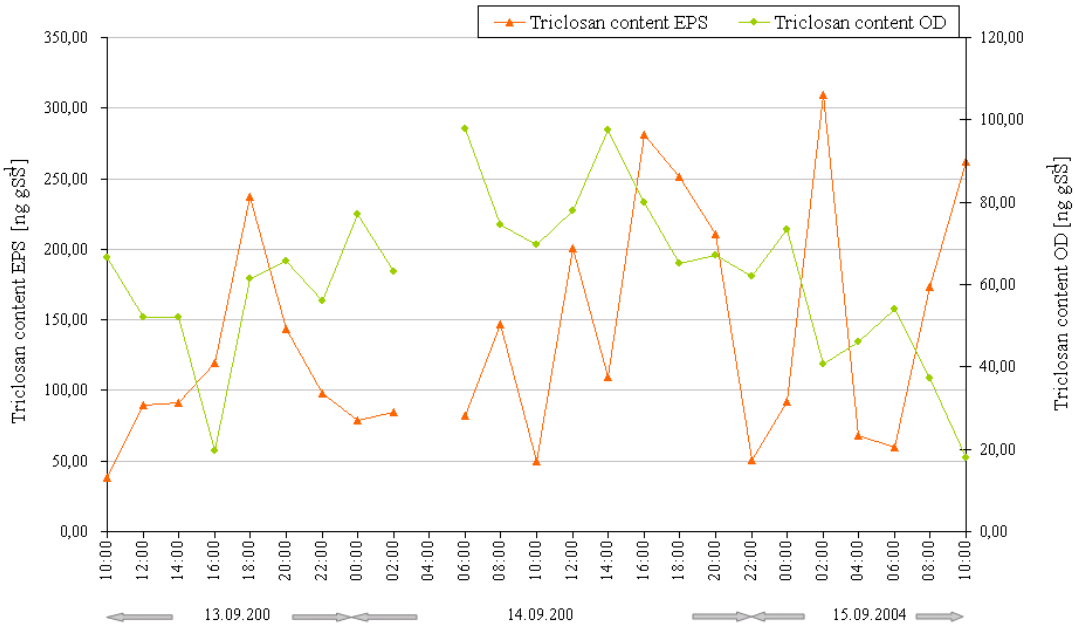


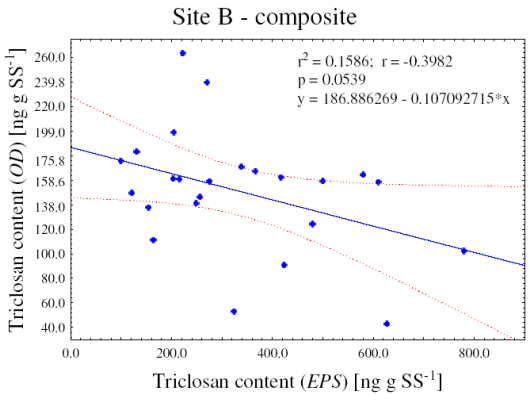
Figure 5.7: Triclosan content in the EPS fraction [ng gSS^{-1} and ng VSS^{-1}]

Figure 5.8 *(a)* compares the content of the EPS and the bulk phase, the oxidation ditch

[ng g SS^{-1}]. According to statistical analyses the content of EPS is in inverse correlation to the content of the liquid phase, even though it is weak (*r=-0.3982, p=0.0539, Figure 5.8 (b) - confidence limits of p=0.05 indicated as red-doted line*).

(a)





(b)

Figure 5.8: *(a)* Triclosan content EPS and Oxidation Ditch (*OD*) [ng gSS^{-1}]; *(b)* Correlation between Triclosan content in EPS and bulk phase of oxidation ditch

Impact on effluent concentration

Referring to section 5.2.1.4.2, where the effluent concentration of Triclosan was significantly higher than the concentration of the bulk phase of the oxidation ditch, one could conclude from Figure 5.9 and Figure 5.10 that these high effluent concentrations might have resulted from redissolved Triclosan within the final clarifier.

The very high effluent values and EPS values are emphasised in these both figures with a red circle. Assuming a certain residence time within the clarifier, high EPS amounts may be one reason for the high effluent concentration.

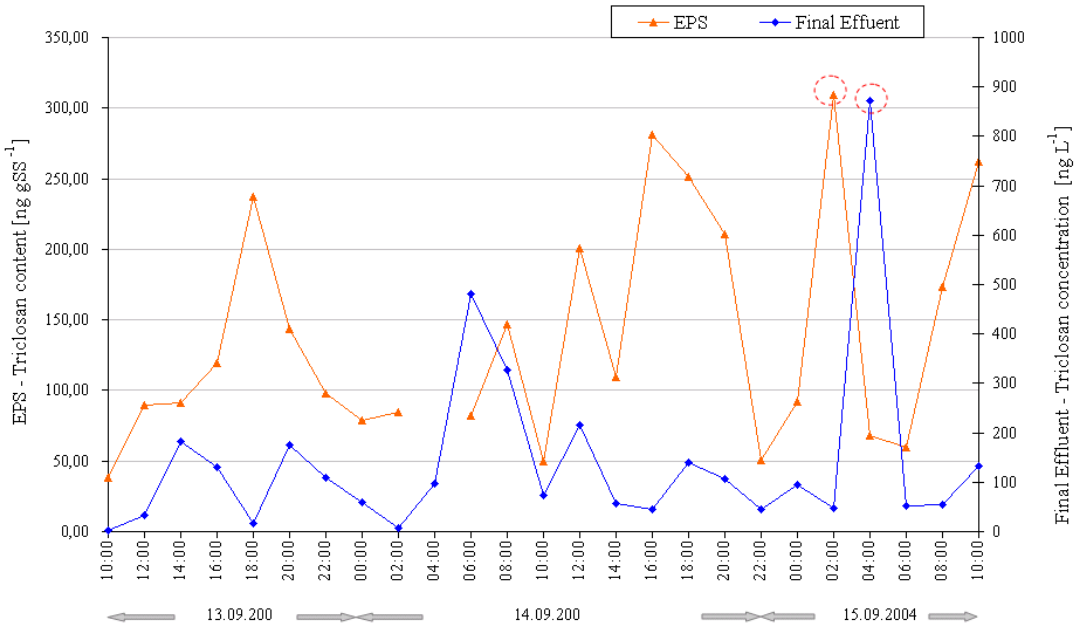


Figure 5.9: Triclosan content EPS [ng gSS^{-1}] and Final effluent concentration [ng L^{-1}]

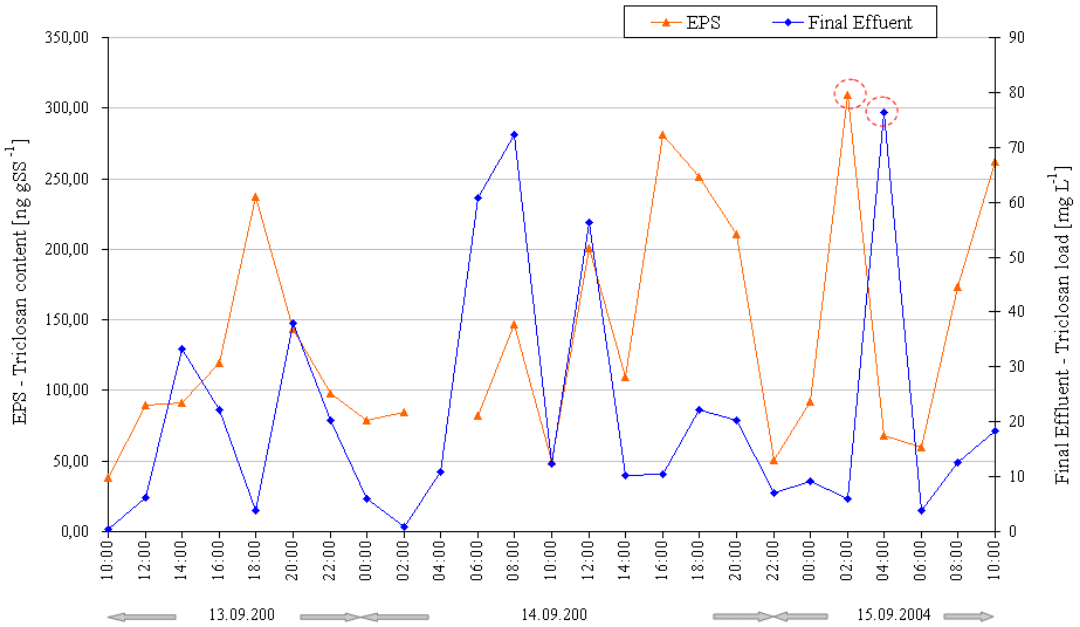


Figure 5.10: Triclosan content EPS [ng gSS^{-1}] and Final effluent load [mg h^{-1}]

In fact, this hypothesis might corroborated by the study conducted by Schneider *et al.* (2004*a*), where a new balancing between solid bond form and dissolved Triclosan was reported within the final clarifying process. This might be verified by regarding the statistical graphs for the correlation of Triclosan EPS concentrations within the sludge samples of the oxidation ditch and the Triclosan effluent concentrations after two hours (*r=0.5387, p=0.0097, n=22, Figure 5.11*).

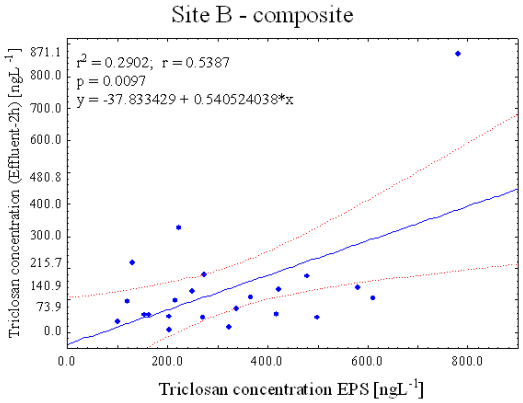


Figure 5.11: Correlation of Triclosan concentration of the EPS fraction to Triclosan concentration of the Final effluent after two hours, *(red-doted lines = confidence limit of p=0.05)*

5.3 Biomass and wastewater characterisations

Wastewater properties such as chemical oxygen demand (COD), pH and temperature are known to impact on process conditions during conventional wastewater treatment. Within this study it was of interest to investigate whether there might be any influences of the wastewater conditions on the removal of trace substances. Therefore, analyses were conducted for COD, suspended solids, proteins and carbohydrates for liquid phases of each sample and where possible for biomass samples.

Biomass characterisation has been undertaken with the collected biomass of Site A (*A5*, fixed biofilm of the rotating biological contactor), the suspended solids from the oxidation ditch of Site B (*B2*), the activated sludge samples of the Site D (*D4* = anoxic lane, *D5* = aerob lane) and the returned activated sludge (RAS, *D6*). No biomass samples were available from the trickling filter of Site C and this WTP will therefore not be mentioned within this section.

One aim of this investigation was to find possible impacts of wastewater conditions on the removal of pharmaceuticals, results will not be evaluated for general WTP conditions, rather for influences on the specific compound removal. The possible impact on Triclosan removal for the whole sampling period was statistically analysed using the data software STATISTICA (StatSoft, Inc.). Confidence limits of $p=0.05$ are indicated in those statistical graphs by red-doted lines.

Liquid phase and biomass characteristics resulted in a high amount of various data. Therefore, the *Result and Discussion* sections include selected data which were found to be significant on the Triclosan elimination. A full scale sampling list of each site, containing all results can be found in the Appendix.

The following sections will provide details of the possible correlations for pH and temperature, soluble COD, soluble proteins and soluble carbohydrates and biomass characteristics, such as lipid content and EPS regarding the fate of Triclosan within wastewater treatment process. Some specific biomass characteristics will be explained in more detail within its section.

The results for the grab samples will be presented as a comparison between the four WTPs, while the 48-hour monitoring sampling will be shown in separate graphs. Results of the grab sampling period have to be handled with care regarding their non-hydraulic flow correlation.

5.3.1 Temperature

The temperature is an important parameter for biomass activity. The elimination of organic substances within wastewater and sludge depends on the temperature. Within this sam-

pling period the temperature of the liquid samples increased with increasing environmental temperature.

As samples of the 48-hour monitoring period has been obtained by an automatic sampler without online temperature measuring, no temperature data are available for this part and correlation of temperature to Triclosan removal can be undertaken only for the grab sampling period. Statistical analysis for correlations between Temperature and Triclosan were determined for each unit process, Triclosan concentrations and removal rates of Triclosan for each treatment stage, and also for the overall removal processes.

5.3.1.1 Grab sampling

Figure 5.12 gives an overview of measured temperature within the grab sampling period, indicating the spectrum of determined temperatures (minimum to maximum value [°C]). Average values over the monitored period are marked by small vertical bars and were connected by trend lines between each treatment step.

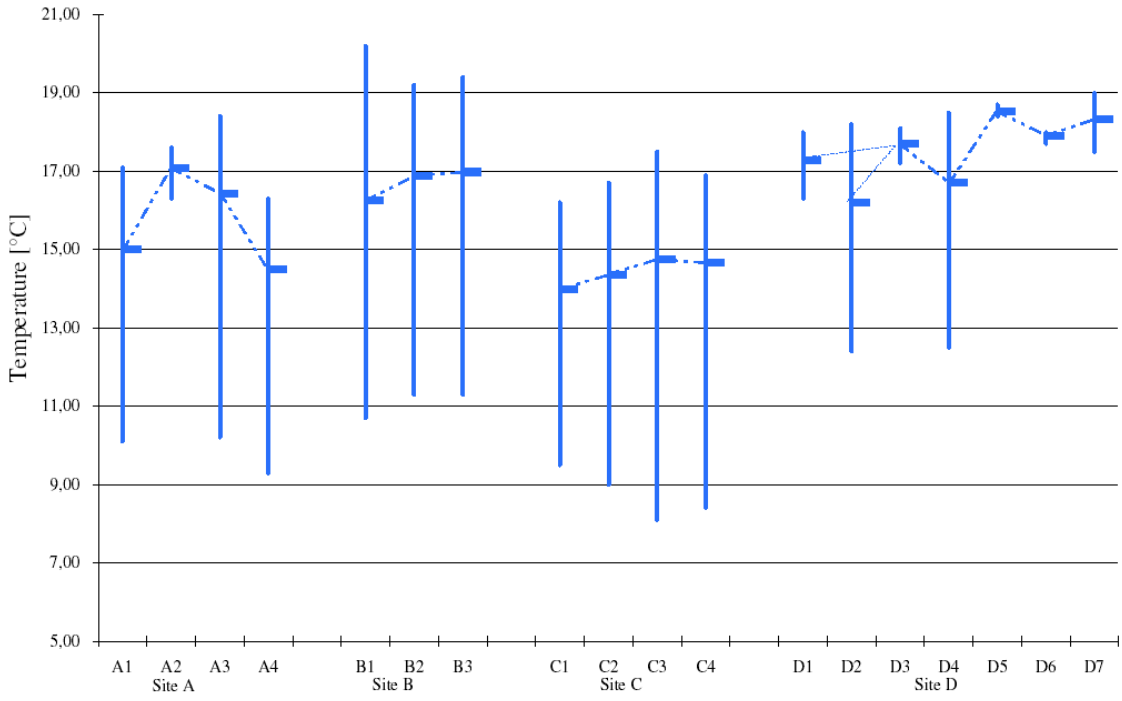


Figure 5.12: Variation of Temperature values - Site A, B, C, D

Site A: Assuming the fact that samples taken from the crude influent (*A1*) and the RBC-effluent (*A3*) can be correlated due to constant positive removal rates, these removal rates have been taken into consideration for statistical analysis. In fact, the rising **temperatures**

demonstrated a significant increase in Triclosan removal between Influent (*A1*) and RBC-effluent (*A2*). No correlation could be determined between influent and effluent treatment steps, most likely due to the lack of Triclosan's hydraulic flow correlation. Figure 5.12 shows the correlation graph between temperature and Triclosan removal (*A1: r=0.9030, p=0.0358; A2: r=0.9585, p=0.010*).

Triclosan concentration in the oxidation ditch of **Site B** decreased with increasing temperature though the correlations are not very significant (*r=-0.7357, p=0.1549*). **Site C** showed clear evidence for lower Triclosan concentrations in the effluent with increasing temperature, with a highly significant correlation for temperature and overall removal (*r=0.9363, p=0.06377*). **Site D** did show as well correlation between the overall removal and temperature (*r=0.9992, p=0.0259*), though it must be stated that the correlation was only undertaken with three data pairs. All correlation graphs can be seen in Figure 5.13.

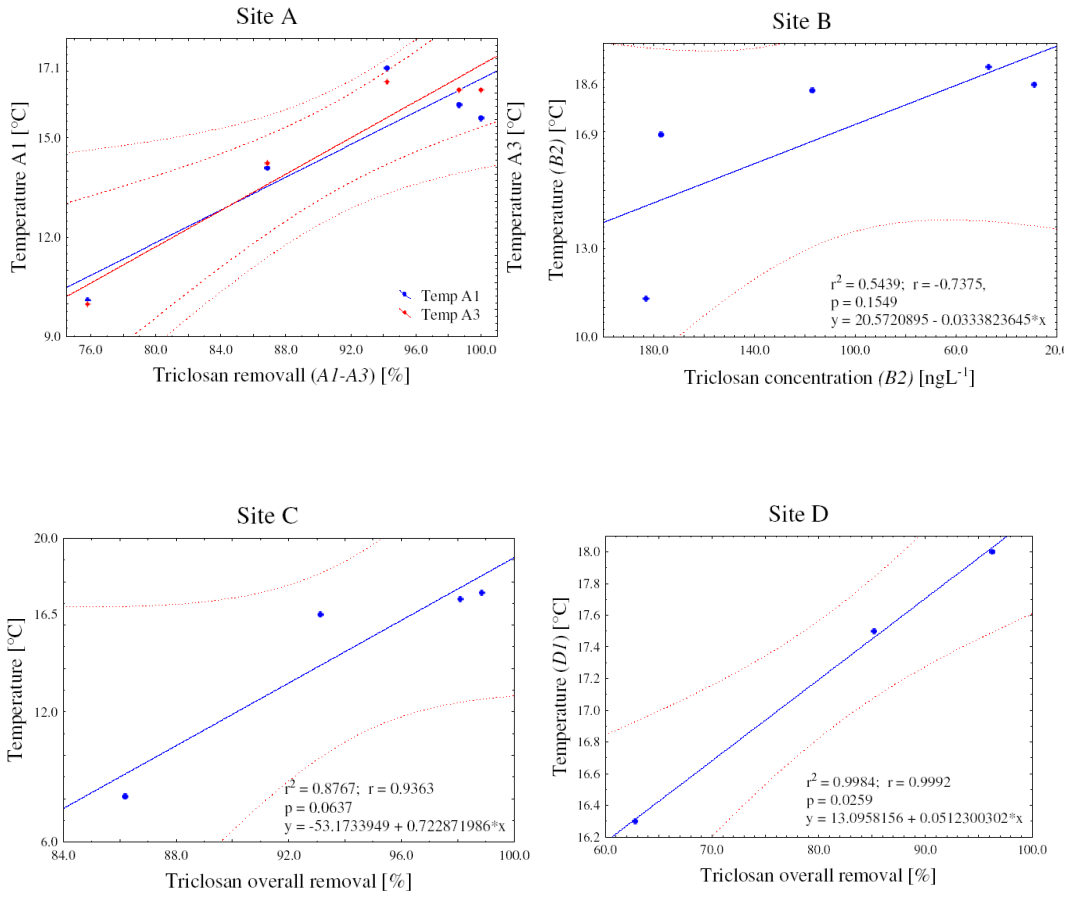


Figure 5.13: Temperature correlated to Triclosan overall removal, concentration resp. - Site A, B, C, D

Assuming ideal conditions and ignoring the fact of the non-hydraulic flow correlating sampling, the results of these correlations might indicate that Triclosan is more readily biodegradable at higher temperatures causing more bioactivity in the biomass.

5.3.2 pH values

The pH is known to have an impact on the fate and removal of the Triclosan compound due to its phenolic character (Lindström *et al.*, 2002; Latch *et al.*, 2003; Mezcua *et al.*, 2004). The pH influences the bioavailability, phototransformability and sorption properties of Triclosan.

Since the pKa is a characteristic constant of the specific analytes from the following definition of the pK_a, $pKa = pH + log\left(\frac{[AH]}{[A-]}\right)$, one can conclude that relative amounts of the neutral and ionic forms of the analytes could be easily adjusted by varying the pH. Moreover, if the pH is approximately two units from the component pK_a more then 99% of the analytes will be either ionic or in a neutral form, depending on the direction of the pH shift. It can therefore be said that Triclosan is predominantly in its neutral form at pH 7.0 and below (*phenolic form, non-dissociated at* $pH < pK_a$) and predominantly in its ionized form at pH 8.5 and above (*phenolate, dissociated form at* $pH > pK_a$) (Orvos *et al.*, 2002).

A compound in its ionic form is more hydrophilic and it not only tends to have less interaction with hydrophobic phase or surface, but also tends to be more solvated with water molecules, whereas the non-ionic form is expected to react more as hydrophobic compound. Triclosan has been found to have a higher tendency to be sorbed at low pH values and to be desorbed at higher pH values (Antusch, 1999; Kuch *et al.*, 2003).

Furthermore, the phototransformation process is reported to be highly dependant on the specification of the Triclosan compound. It has been shown that the ionic form of Triclosan is more easily transformed into its derivatives. Figure 5.14 shows the properties of pH depending form of Triclosan (Lindström *et al.*, 2002). Mezcua *et al.* (2004) reported the phototransformation into 2,7/2,8-dichlorodibenzo-*p*-dioxin at higher pH values due to the overlapping of the UV spectra with the solar spectra. The wavelength spectra can also be seen in Figure 5.14.

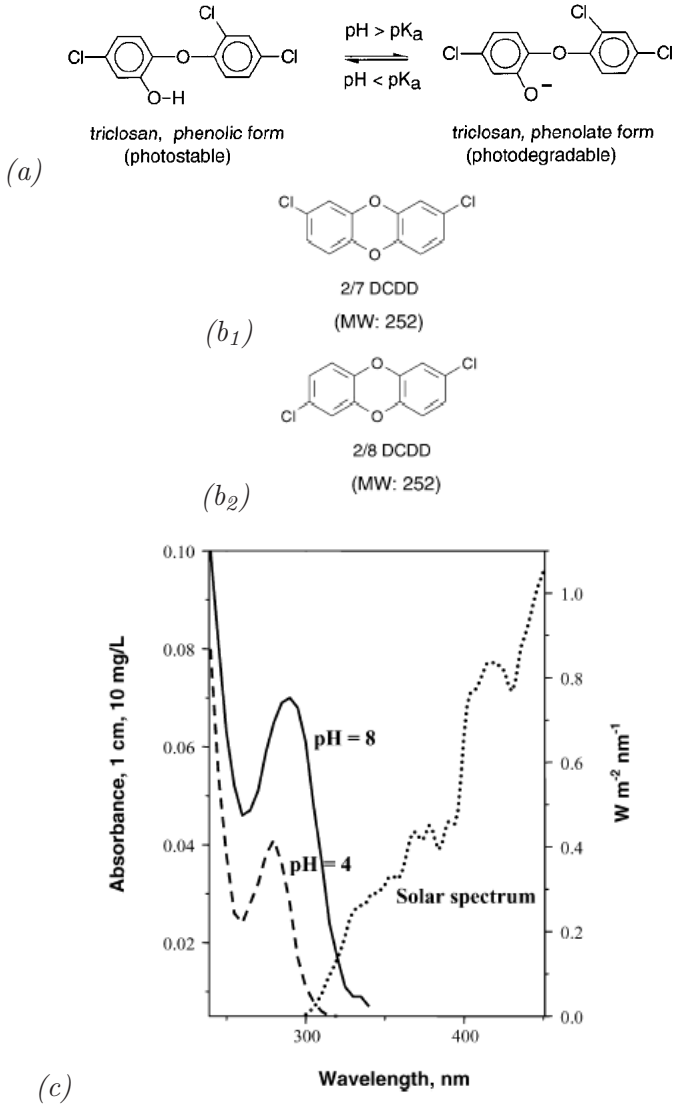


Figure 5.14: *(a)* pH depending forms of Triclosan (Lindström *et al.*, 2002), *(b_1)* 2,7- and *(b_2)* 2,8-dichlorodibenzo-*p*-dioxin and *(c)* wavelength spectra for Triclosan at different pH and sunlight UV spectra (Mezcua *et al.*, 2004)

Within wastewater treatment processes Mezcua *et al.* (2004) observed a simultaneous disappearance of Triclosan and the appearance of the dioxin depending on the pH and the organic matter content. Consequently, it can be said that with varying pH of the aquatic media, the form of the Triclosan compound changes and therefore its properties and chemical behaviour.

5.3.2.1 Grab samples

The measured pH values of the grab samples varied widely and are represented for each step of the sites as maximum, medium and average values in Figure 5.15, with trendline connections between treatment stages.

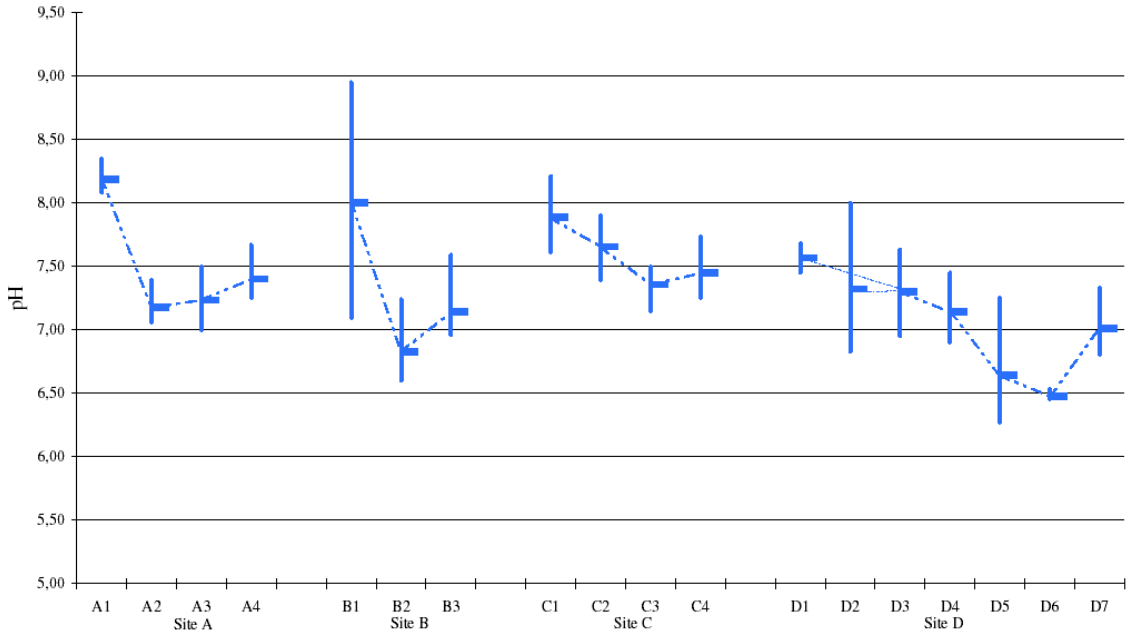


Figure 5.15: Variation of pH values - Site A, B, C, D

As no detection method existed for derivatives of Triclosan within this study, the analyses for pH influences could only be undertaken according to its removal in the wastewater treatment plant and no evaluation could be carried out for possible degradation products. In Figure 5.16 the correlation graphs of Site A - D are shown and will be explained in the following section.

According to the assumption for **Site A**, using the overall removal between crude influent (*A1*) and RBC-effluent (*A3*) as basis for interdependence, the pH of the RBC-Influent (*A2*) showed a significant correlation to Triclosan removal between crude Influent (*A1*) and RBC-effluent (*A3*) *(r=-0.9868, p=0.0132* - correlation graph can be seen in 5.16*)*. One possible explanation may be due to the properties of Triclosan with respect to pH changes. Assuming that sampling spot A2 represents more less the settling point between the two observed removal rates, Triclosan is more likely to be found in the liquid phase at higher pH, subsequently decreasing the removal. However, this statement might be relatively vague as pH values have not dramatically exceeded pH 7.0 and might be caused by pH changes at (A1), which is significant above the pH '*threshold value*' for Triclosan's ionised form.

However, the correlation of pH (*A2*) with Triclosan concentration (*A2*) was also found to correlate, even though this was not very significant *(r=-0.8664, p=0.134)*. On the other hand, it has to be admitted that the pH is dependent on temperature, with pH in this study shown to decrease with increasing temperature.

Site B showed, for its concentration of Triclosan within the liquid phase of the oxidation ditch, a significant increase with increasing pH (*B2*) *(r=0.8400, p=0.0180)*. This could indicate the more hydrophilic properties of Triclosan with increasing pH, even though the pH did not significantly exceed pH 7.0 and the significance of this correlation is due to one single data point (*pH = 7.2,* $c_{Triclosan}$*= 943 ngL*$^{-1}$). However, removal rates between influent (*B1*) and oxidation ditch (*B2*) clearly increased with decreasing pH of the oxidation ditch *(r=-0.9457, p=0.0013)*, showing evidence for Triclosan's tendency to be more easily sorbed to suspended solids at lower pHs and therefore increase removal rates.

The values of pH varied more widely for the primary settling tank (*C2*) of **Site C**, showing significant higher Triclosan concentrations in the liquid phase at higher pH *(r=0.8567, p=0.00637)* and no influence on pH due to temperature changes were observed.

Within the activated sludge plant of **Site D**, a decreasing pH of the anoxic zone (*D4*) was observed, which clearly increased the removal within the activated sludge plant (*D4-D5*). The highest observed overall removal could be related to the lowest observed pH value, which was significantly below the *'pH threshold value'* for Triclosan. As Triclosan could be detected just in one sample of the returned activated sludge, no statistical analysis could be conducted for that particular sludge type.

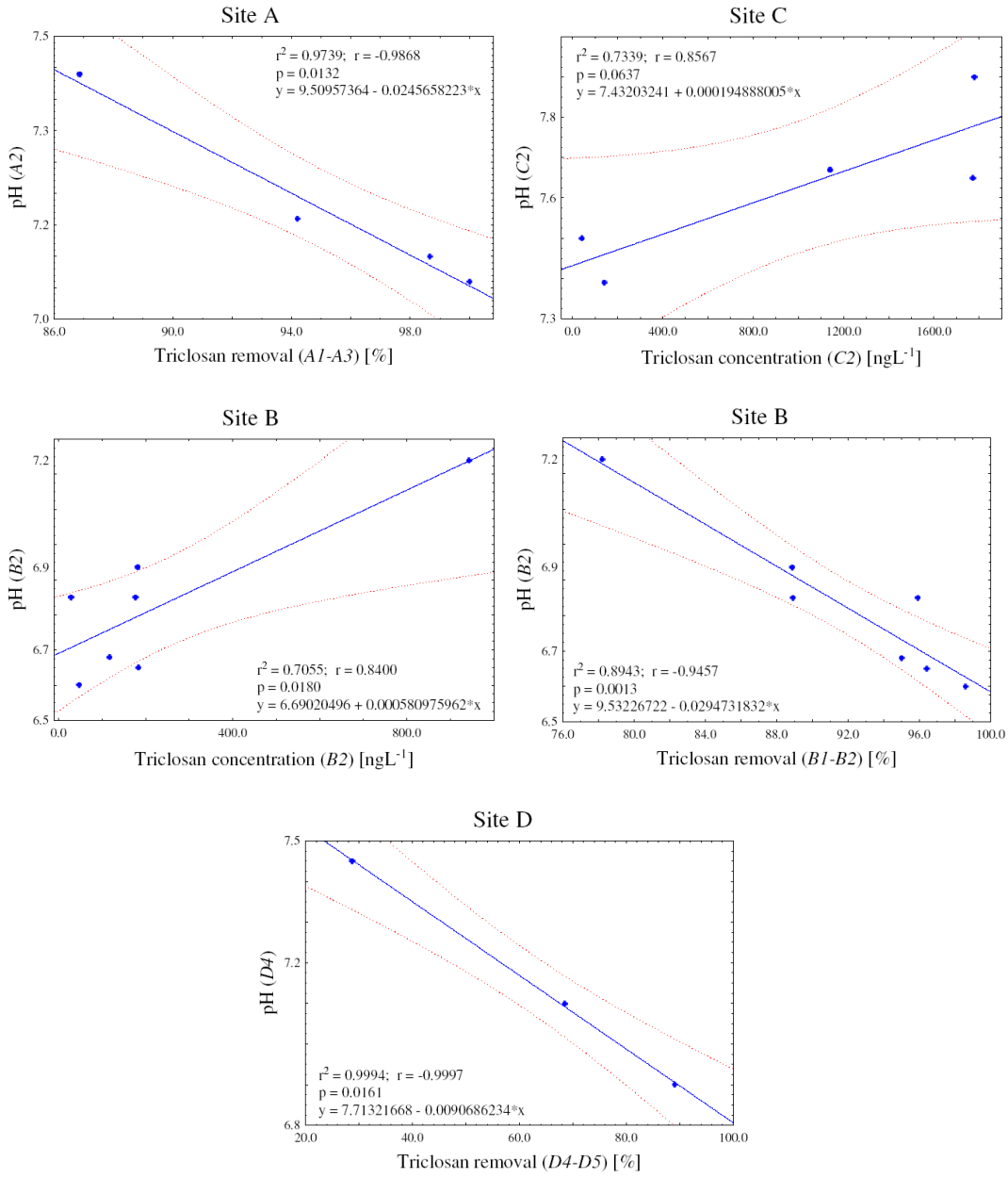


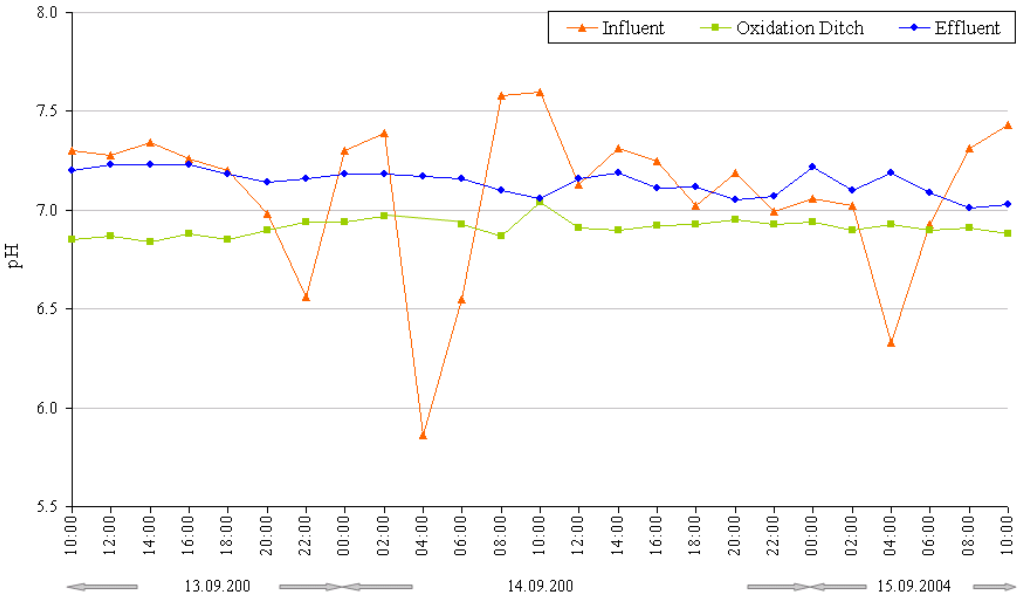
Figure 5.16: Correlation between Triclosan removal, concentration respectively and pH

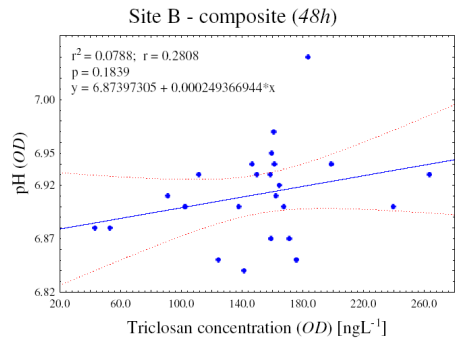
5.3.2.2 48-hour monitoring sampling

Within the 48-hour monitoring sampling no relevant correlation could be observed between prevalent pH and Triclosan concentrations or Triclosan removal rates. This may have been due to the fact that pH measurements could not be determined immediately after the samples collection, but were measured up to 24h hours later. This may have resulted in pH changes, especially within the activated sludge samples. On the other hand it has to

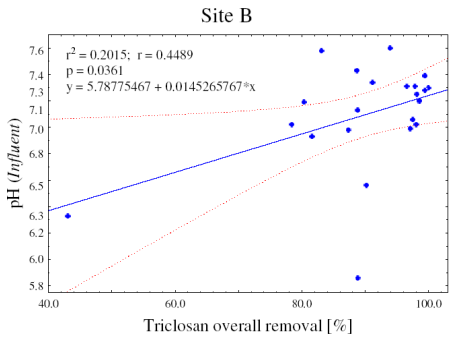
be admitted that no widely pH changes within the bulk phase of the oxidation ditch has been observed, whereas, interestingly, slight correlation could be found between the overall removal of triclosan and the pH of the influent. This might be indicating the influence of pH changes on the removal rates of Triclosan. Figure 5.17 *(a)* shows the pH variation of the 48-hour monitoring sampling. The extreme influent pH values could be a result of industrial influents, but showed no extreme values of Triclosan concentration. From Figure 5.17 *(b)* it can be seen that pH and Triclosan concentrations within the oxidation ditch show a slight tendency, although this can not be proved by statistical correlation..

(a)





(b₁)



(b₂)

Figure 5.17: (a) pH values (48-hour monitoring) and (b_1) correlation between Triclosan concentration and pH (oxidation ditch); (b_2) correlation between Triclosan overall removal and pH (influent)

There was also no observed direct influence between the pH of the oxidation ditch and the sorbed amount of Triclosan to the **extracellular polymeric substances**. This is in accordance to the observation within the bulk characteristics, where no correlation could be found between prevalent pH values and concentration of Triclosan in the liquid phase of the oxidation ditch. Changes of pH values within the wastewater flow of the treatment work might have an important impact on the prevalence of Triclosan in either bound or dissolved form, but this cannot be concluded definitively on the basis of this study.

5.3.3 Chemical Oxygen demand (COD)

The chemical oxygen demand (COD) is a value in water analysis representing a summary load of contaminants, indirectly used for the amount of organic substances. Its overall removal is used within wastewater treatment processes as indicator of efficiency and as an indicator for the biological removal capacity for the type of wastewater. The COD has been conducted to investigate if a high concentration of the determined pharmaceutical compound might adversely affect the COD removal of the WTPs. In addition, it was also of interest to see if a high removal of COD could be correlated with a high pharmaceutical removal as found by Federle *et al.* (2002) for conventional activated sludge (CAS) experiments.

5.3.3.1 Grab sampling

Figure 5.18 shows the distribution of the measured COD results (mgL^{-1}) of each sampling step indicating the spectrum of determined COD values (minimum to maximum value). Average values over the monitored period are marked by small vertical bars. For a better visualisation of increasing or decreasing values within the wastewater treatment process, the average values between each treatment step have been connected with trend lines. Minimum, maximum and average COD concentrations and average COD overall removal for the grab sampling period are given in Table 5.6.

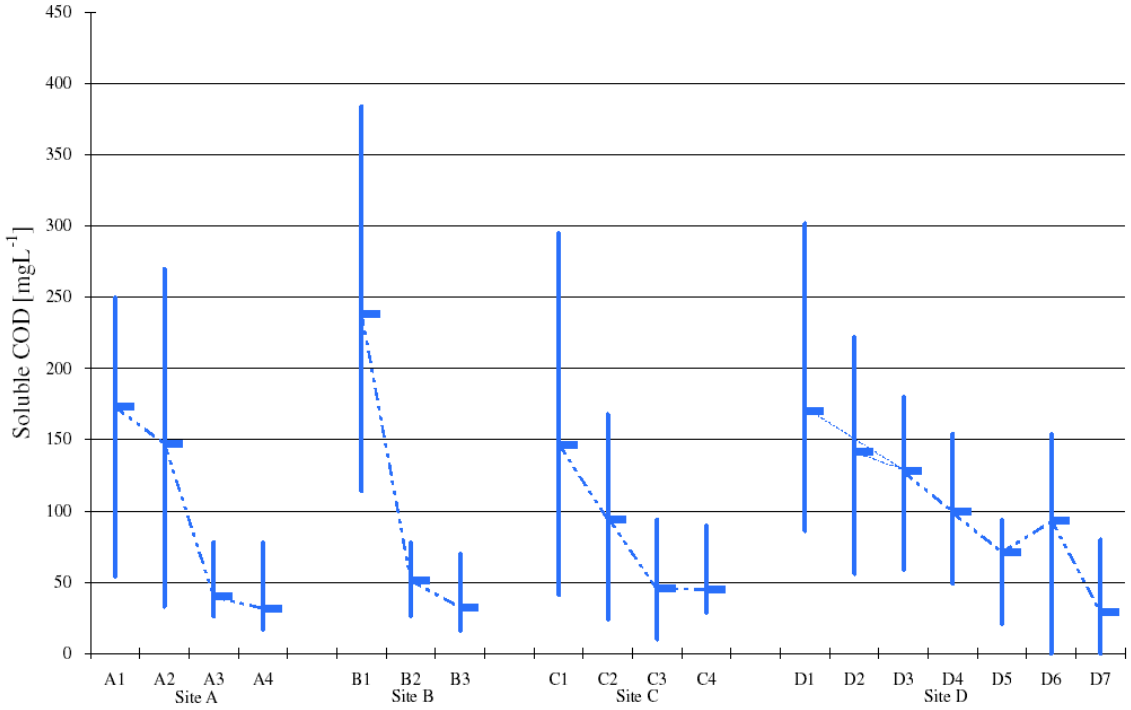


Figure 5.18: Variation of COD values [mgL^{-1}] - Site A, B, C, D

Table 5.6: COD concentrations and overall removal - Comparison grab sampling period

	COD concentration [mgL^{-1}]		
Wastewater Treatment Plant	Influent	Effluent	COD overall removal [%]
Site A (Rotating Biological Contactor)	54 (min) 250 (max) 173 (med)	17 (min) 78 (max) 31 (med)	68 (min) 90 (max) 78 (med)
Site B (Oxidation Ditch)	114 (min) 384 (max) 239 (med)	16 (min) 70 (max) 33 (med)	81 (min) 89 (max) 86 (med)
Site C (Trickling Filter)	41 (min) 295 (max) 146 (med)	29 (min) 90 (max) 45 (med)	44 (min) 84 (max) 74 (med)
Site D (Activated Sludge Plant)	86 (min) 302 (max) 170 (med)	>10 (min) 80 (max) 34 (med)	44 (min) 91 (max) 74 (med)

Statistical analysis for correlations between COD and Triclosan concentrations were determined for each unit process and concentration values and also for the overall removal processes.

Within this study overall **COD** removal showed no correlations for **Site A** (*r=0.3290, p=0.524, n=6*). There was also no correlation with either Triclosan concentration or Triclosan removal for any of the sampling stages. Similar conditions have been found for **Site B** (*r=-0.4755, p=0.2808, n=7*) and **Site D** (*r=-0.4943, p=0.5057, n=4*). **Site C** has been the only WTP where values of the taken sample correlated significantly (*r=0.9618, p=0.0089, n=5*). The correlation graphs are shown in Figure 5.19.

However, the values relating only to WTP C and not to WTP A, B, D ought to be handled with care as COD values depend on daily variations and hence results might be influenced by the sampling stratification of the grab sampling period. Furthermore COD measurements within the grab sampling period could not be undertaken in triplicates but just once per sample reducing project expenses. This lead to the risk of large deviations for each analysis.

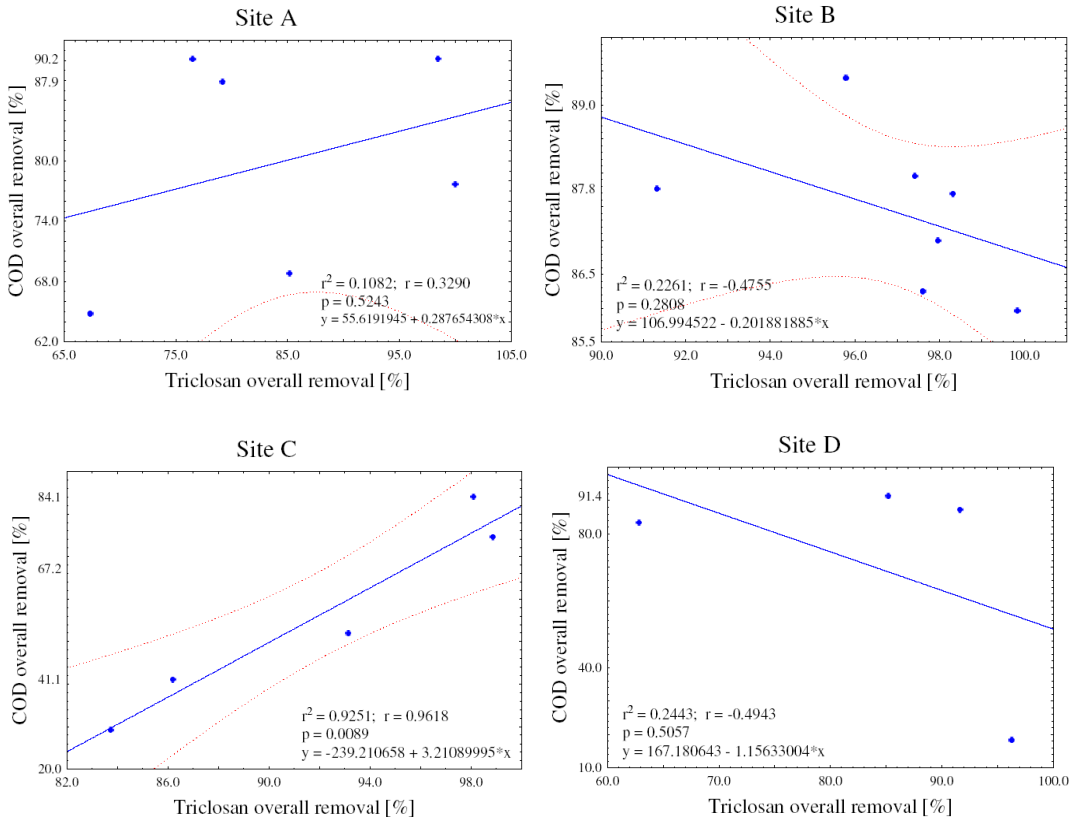


Figure 5.19: COD overall removal correlated to Triclosan overall removal - Site A, B, C, D

5.3.3.2 48-hour monitoring sampling

COD concentrations for the 48-hour monitoring sampling varied from 83 to 386 mgL^{-1} for influent, from 19 to 51 mgL^{-1} for the oxidation ditch and from 19 to 34 mgL^{-1} for the effluent. Data are shown in Figure 5.20.

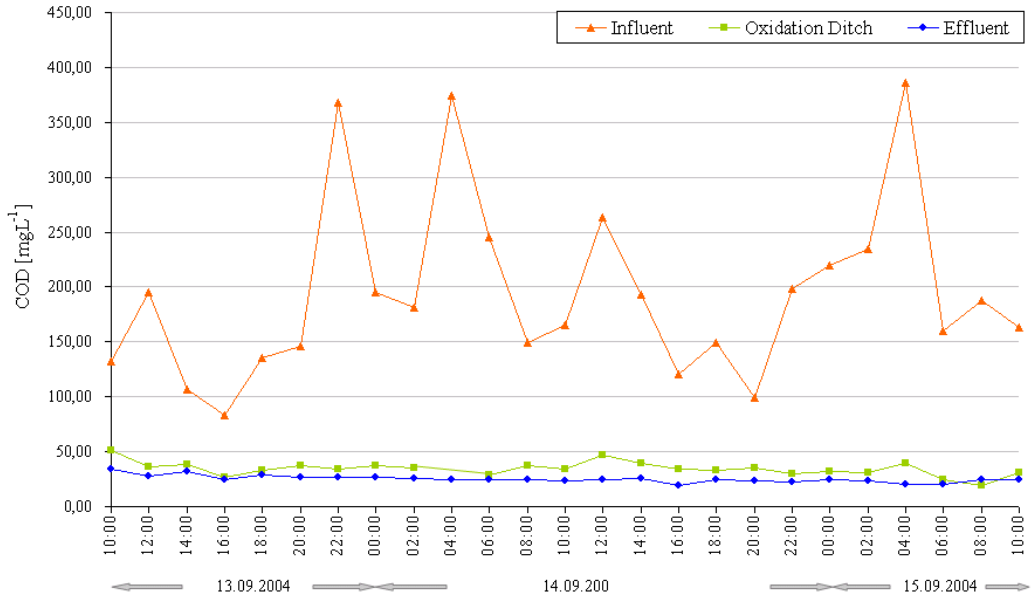


Figure 5.20: COD values - Site B - 48-hour monitoring sampling

The 48-hour monitoring sampling for Site B made it possible to analyse liquid and biomass characteristics within the hydraulic retention time. Data for both parameters (Triclosan and COD) were analysed statistically and compared for each treatment stage regarding concentrations, primary and secondary removal rates and also for overall removal rates. Furthermore, concentrations and removal rates were analysed regarding possible hydraulic flow differences.

Comparison of data for COD concentration and Triclosan concentration for influent, oxidation ditch and effluent lead to a weak correlation of $R^2 = 0.4701$ as can be seen in Figure 5.21. In fact, this comparison of data in such clusters does not give any evidence for time and flow dependency, especially for two parameters which are known to be both removed within the wastewater process.

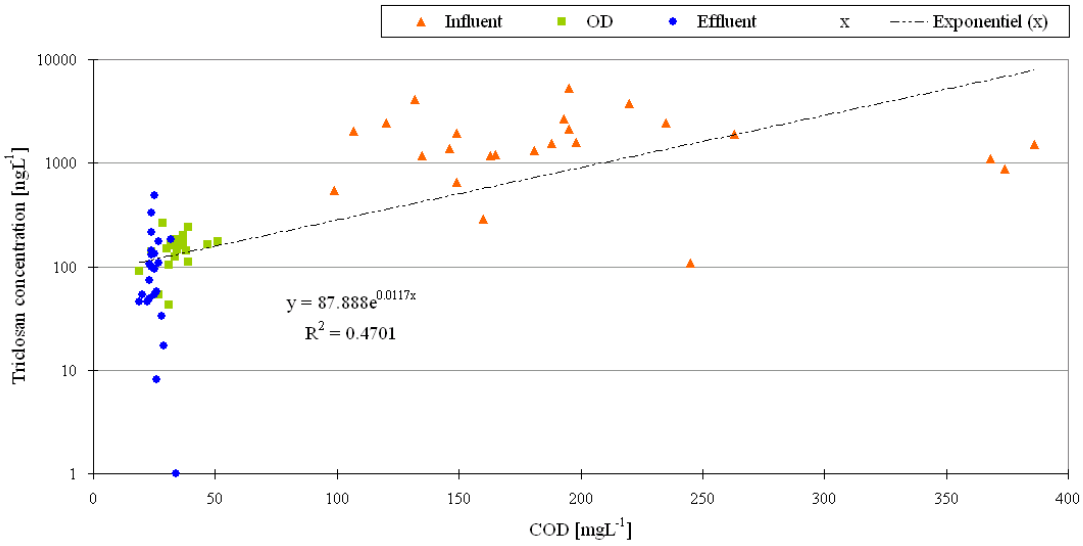


Figure 5.21: COD concentration vs Triclosan concentration - Site B - 48 hour monitoring

Comparing the concentrations of the liquid phase for influent and effluent did not reveal in any significant correlations *(see Figure 5.23)*, neither could any correlation be found between Triclosan removal and COD removal for either primary *(Inf-OD)*, secondary *(OD-Eff)* or overall removal *(Inf-Eff)*.

In fact, the correlation of overall removal rates (none hydraulic flow correlated and hydraulic flow correlated - *assumed 30h HRT*) showed a negative correlation as opposed to the expected positive correlation. Both graphs can be seen in Figure 5.22. The negative increase in graph 5.22 can be attributed to one anomalous value for a effluent sample (marked with a red circle). Without this value, the overall removal of Triclosan would vary between 78 and 99% *(respectively 85 to 96% for assumed 30h HRT)*, regardless the COD overall removal.

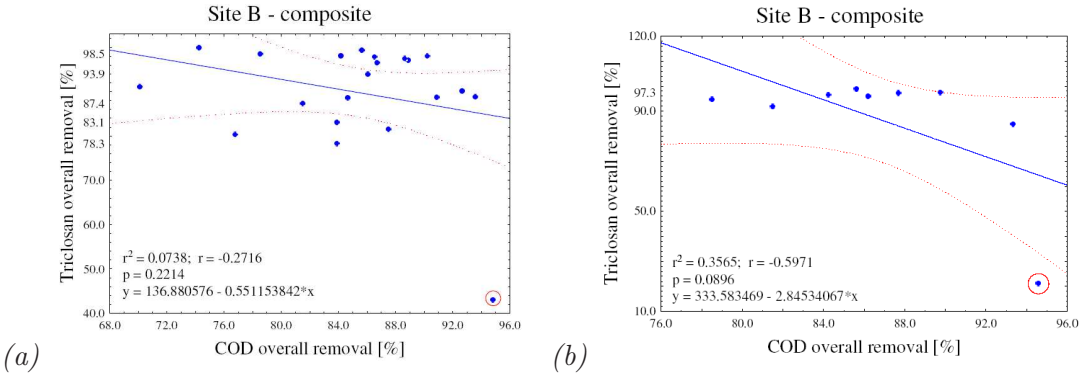


Figure 5.22: *(a)* COD overall removal vs Triclosan overall removal and *(b)* COD overall removal vs Triclosan overall removal *(hydraulic flow correlated - 30h)* - Site B - 48-hour monitoring sampling

Therefore, it might be concluded that COD overall removal efficiency within this monitored full scale WTP did not show any significant influence on the Triclosan removal efficiency as it has been stated for laboratory CAS-experiments by Federle *et al.* (2002).

Nevertheless, COD concentrations of the oxidation ditch seemed to have impact on the Triclosan concentration within the oxidation ditch. Furthermore, Triclosan uptake within the EPS fraction and therefore Triclosan removal from the liquid phase seemed to be influenced by COD concentrations of the bulk phase (OD) and of the biomass (sludge). For a better understanding this will be explained in the following section with the statistical correlation graphs.

Figure 5.23 shows the correlated concentration for COD and Triclosan for each treatment unit. It can be seen that there are no correlations for influent (*r=-0.1023, p=0.634, n=24*) and effluent (*r=-0.2772, p=0.190, n=24*) and just a very weak correlation for the oxidation ditch (*r=0.3512, p=0.0934, n=24*).

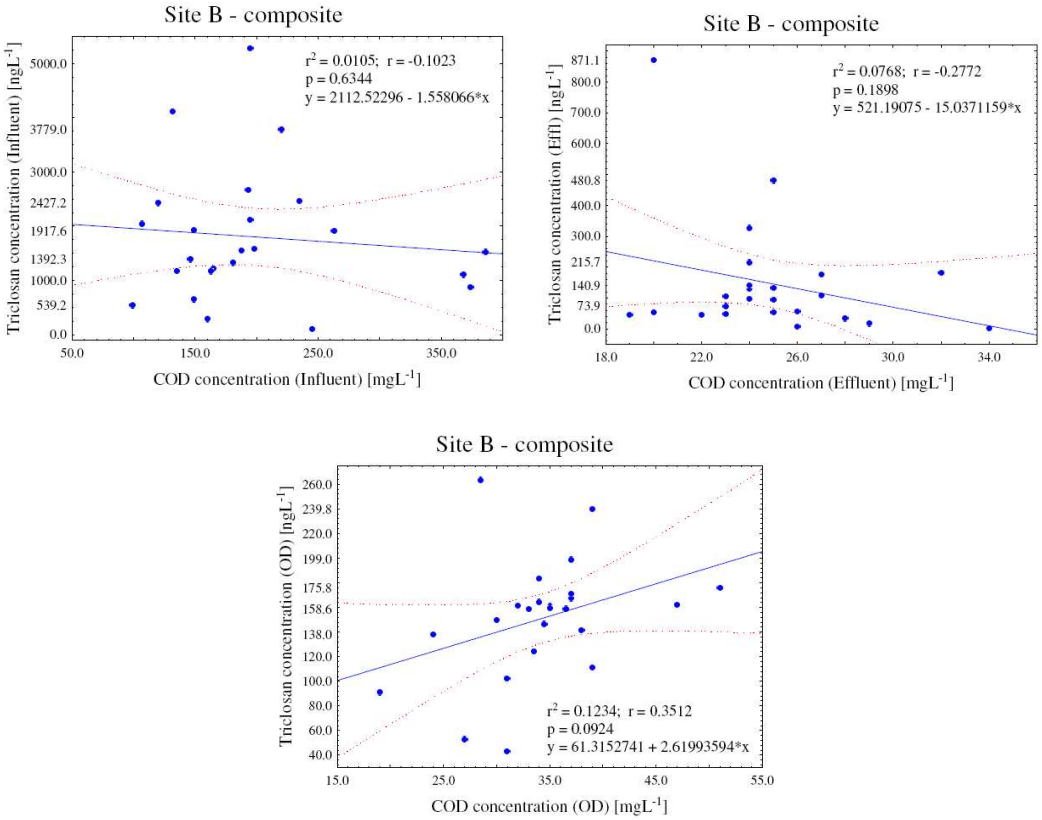


Figure 5.23: Correlation of COD concentrations and Triclosan concentrations for Influent, Effluent, OD - Site B - 48-hour monitoring sampling

The correlation between the COD concentration and Triclosan concentration within the oxidation ditch becomes more interesting if looking at the found Triclosan content within the EPS fraction.

Figure 5.24 *(a)* shows the correlation between the COD concentration in the liquid phase of the oxidation ditch and the EPS Triclosan content (*r=-0.1417, p=0.5090, n=24*), whereas 5.24 *(b)* indicates the correlation for COD concentration in the liquid phase of the oxidation ditch and the Triclosan EPS content after 2 hours (*r=-0.4066, p=0.0604, n=22*).

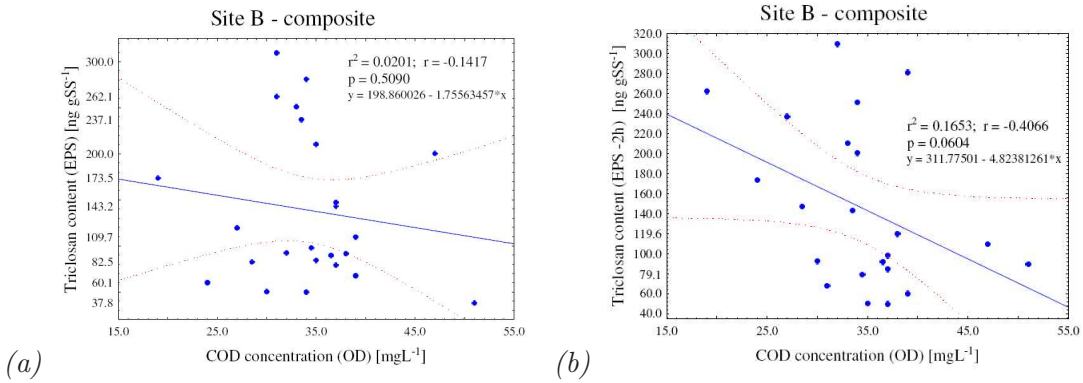


Figure 5.24: Correlation of COD concentrations (OD) and Triclosan content within the EPS fraction - Site B - 48-hour monitoring sampling , *(a)* COD (OD) vs Triclosan content EPS, *(b)* COD (OD) vs Triclosan content EPS after 2 hours

Assuming a two hours duration for the biomass to react to changes within the liquid phase, one could conclude that low COD concentrations within the liquid phase enhance the uptake of Triclosan within the EPS fraction, even though the found correlation is not very significant (*r=-0.4066, p=0.0604*).

Similar conclusions can be drawn from comparing COD influent concentrations to Triclosan concentration within the liquid phase of the oxidation ditch after 2 hours, where higher COD concentrations within the influent seemed to result in higher Triclosan concentration within the liquid phase of the oxidation ditch (*r=0.4352, p=0.0380, n=22* - see Figure 5.25).

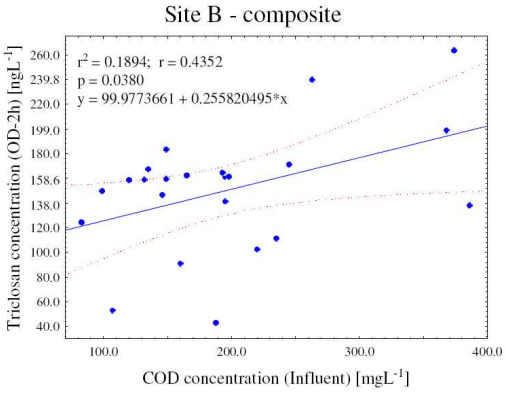


Figure 5.25: Correlation of COD concentrations (Influent) to Triclosan concentration within the oxidation ditch after 2 hours

Similar conclusions can be drawn from comparing COD concentration within the biomass from the oxidation ditch (sludge) with the Triclosan content of the EPS fraction, which

resulted in a moderate correlation (*r=0.5435, p=0.0061, n=24*) as it can be seen in Figure 5.26.

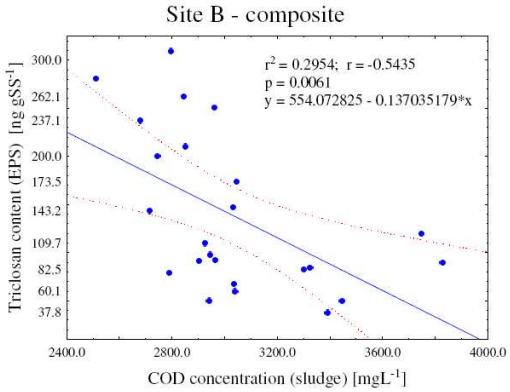


Figure 5.26: Correlation of COD concentration for the biomass (sludge) to Triclosan content within the EPS fraction

From Figures 5.23 to 5.26 it has been shown that higher COD concentrations within the bulk phase (Influent or OD) seemed to result in higher Triclosan concentrations in the bulk phase of the oxidation ditch, whereas lower COD concentrations in the bulk phase resulted in higher Triclosan content within the EPS fraction. Furthermore, lower COD concentration of the biomass seemed to enhance Triclosan uptake within the EPS. These observations might be suggested to the fact that Triclosan can be used by the biomass as an additional carbon donor as it has been reported by Hay *et al.* (2001); Hundt *et al.* (2000).

This phenomenon might explain also why no correlations could be found for primary COD removal and primary Triclosan removal. Assuming on the one hand a high COD concentration for the influent which might lead to a high primary COD removal, but not consequently to high primary Triclosan removal. Whereas on the other hand a low COD influent concentration may result in a lower COD removal rate, but higher Triclosan uptake within the biomass, EPS fraction respectively, resulting therefore in a higher Triclosan removal.

However, it should be noted that these conclusions could just be drawn from one sampling occasion, the 48-hour monitoring period, and it should also be noted that results might be influenced by other uncontrolled wastewater parameters. So, for instance, it has to be admitted that the determination method of COD represents the quantity of possible chemical oxidation without nitrogen compounds, but includes due to meassuring method inorganic compounds, such as iron(II), sulfite, sulfide, and nitrite compounds. Furthermore, results for removal rates of primary and secondary stage might be also impacted by the sampling stratification, location of sampling points in particular.

5.3.4 Total Organic Carbon - *Colloidal Organic Carbon*

Total organic carbon (TOC) is used as reference scale in wastewater treatment processes as indicator for trends in wastewater quality. TOC is a summarised value for dissolved organic carbon (DOC) plus particulate organic carbon (POC). **DOC** is, according to EN 1484, the sum of organically bound carbon which will pass through a membrane filter of pore size 0.45 μm. As the TOC has been measured within this study from the filtered supernatants of liquid samples, with a pore diameter of 1 - 1.5 μm and the results therefore represent the content of **colloidal organic carbon** (COC) rather than TOC or DOC. Nevertheless, the results will be taken as TOC in the following discussion in order to keep a level of consistency.

No measurement has been possible for total organic carbon content of biomass matrix. TOC measurements for liquid samples were only undertaken once for the grab samples, thus representing no significant value and will therefore not be regarded within this discussion.

5.3.4.1 48-hour monitoring sampling

TOC values of influent, oxidation ditch and effluent can be seen in Figure 5.27. Even though both parameters, TOC concentration and Triclosan concentration, show a decrease through the wastewater treatment processes, no significant correlation has been found between the TOC of the liquid samples and the concentration or removal of Triclosan within the liquid samples.

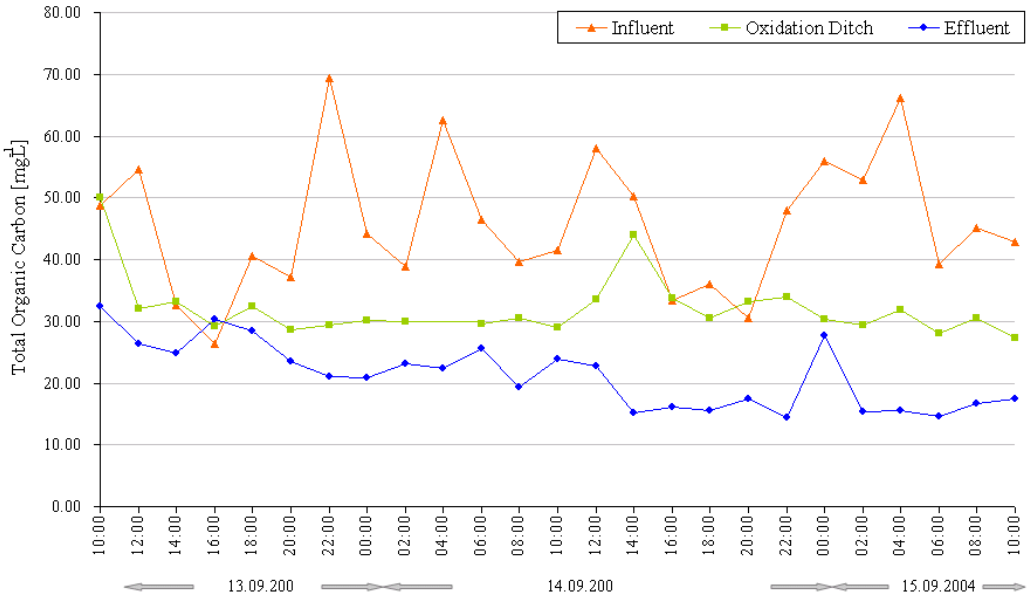


Figure 5.27: Total Organic Carbon concentration - Site B - 48 hour monitoring

Compared to COD values the TOC concentration varied more widely in particular within the oxidation ditch and the effluent. It should be noted that TOC measurements could not be undertaken in triplicates and therefore results ought to be handled with care.

Nevertheless, comparing TOC concentrations to Triclosan concentrations over the whole wastewater treatment process resulted in a correlation of R^2=*0.4424* as can be seen in Figure 5.28. Again this way of comparison does not allow any conclusions about the correlation between concentration of the two parameter in each treatment process or between the elimination rates for both parameters.

Correlations for Triclosan concentrations to TOC concentrations for Influent (*r=0.2707, p=0.2571, n=24*), Effluent (*r=-0.1381, p=0.520, n=24*) and Oxidation Ditch (*r=0.3490, p=0.0946, n=24*) can be seen in Figure 5.29.

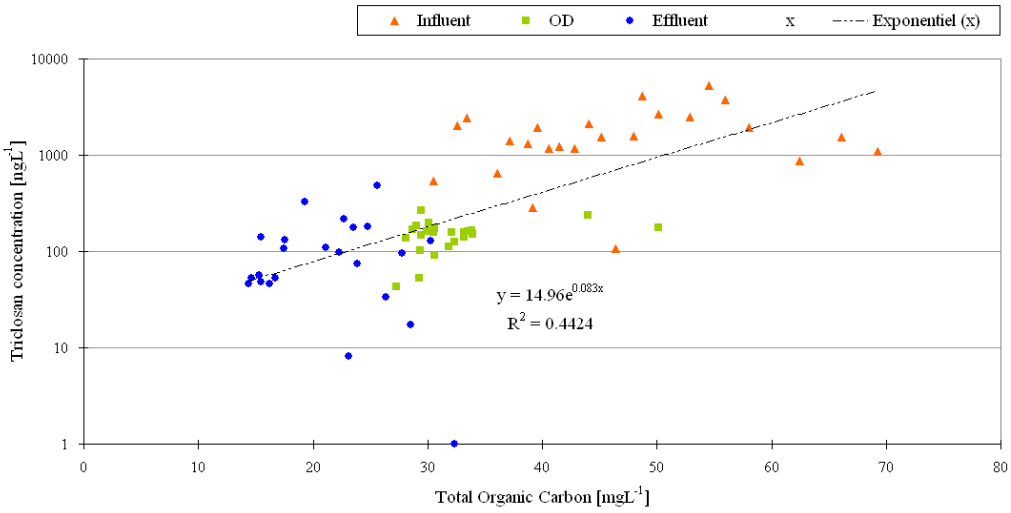


Figure 5.28: TOC concentration vs Triclosan concentration - Site B - 48 hour monitoring

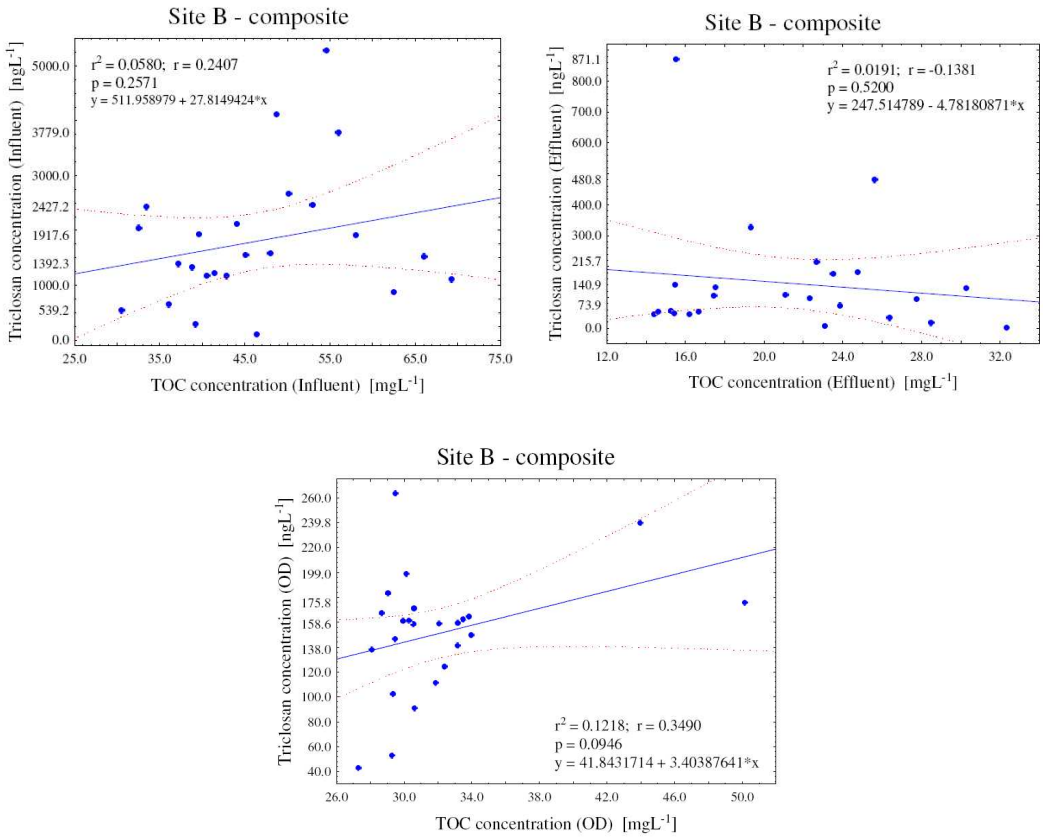


Figure 5.29: Correlation of TOC concentrations and Triclosan concentrations for Influent, Effluent, OD - Site B - 48-hour monitoring sampling

Comparison of removal rates of Triclosan and TOC for primary, secondary and overall removal yielded in no significant correlation. The statistical analysis graph for overall removal of TOC to overall removal rate of Triclosan (*r=-0.3303, p=0.1333, n=24*) is given in Figure 5.30.

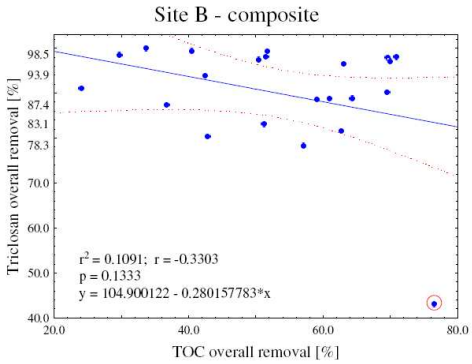


Figure 5.30: TOC overall removal vs Triclosan overall removal - Site B - 48-hour monitoring sampling

As it can be seen from Figure 5.29 to Figure 5.30, TOC removal rates, concentrations respectively, do not allow any precise conclusion about the fate of Triclosan within wastewater treatment processes.

However, the measured TOC parameter is getting more interesting if comparing determined Triclosan concentrations of the oxidation ditch and Triclosan content within the EPS with the specific oxygen demand*.

The **specific oxygen demand** is defined as the ratio of chemical oxygen demand to dissolved organic carbon (COD/DOC). As no DOC was determined within this study, but the TOC was measured from filtrated supernatants, TOC results were taken into consideration for comparing ratios of **COD/TOC** as specific oxygen demand*.

Figure 5.31 shows the statistical analysed graphs for ratio of COD/TOC of the oxidation ditch correlated to Triclosan concentrations of the oxidation ditch (*5.31 (a), r=0.1284, p=0.5498, n=24*) and correlated to Triclosan concentrations of the oxidation ditch after two hours (*5.31 (b), r=0.5535, p=0.0075, n=22*).

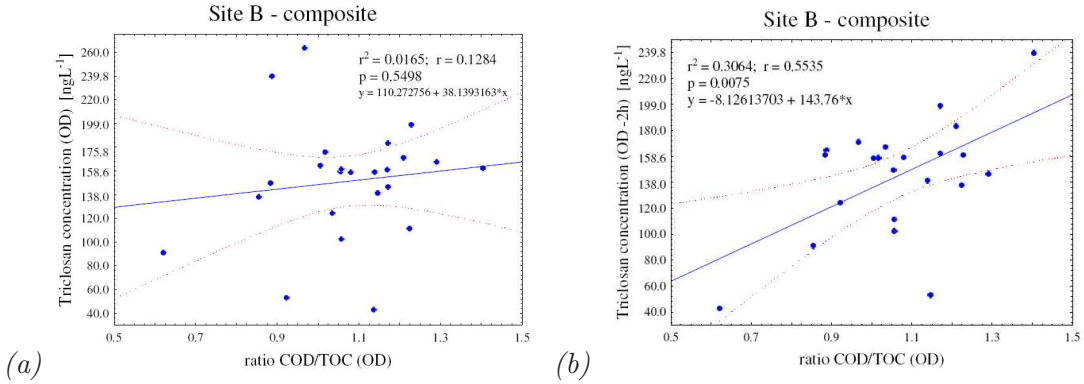


Figure 5.31: Correlation of COD/TOC *(specific oxygen demand*)* and Triclosan concentration - Site B - 48-hour monitoring sampling , *(a)* COD/TOC (OD) vs Triclosan concentration (OD), *(b)* COD/TOC (OD) vs Triclosan concentration after 2 hours (OD-2h)

Correlations between the specific oxygen demand* of the bulk phase of the oxidation ditch (COD/TOC (OD)) and the Triclosan content of the EPS (*(a), r=0.0252, p=0.9070, n=24*), and the Triclosan content of the EPS after two hours (*(b), r=-0.5301, p=0.0112, n=22*) are shown in Figure 5.32.

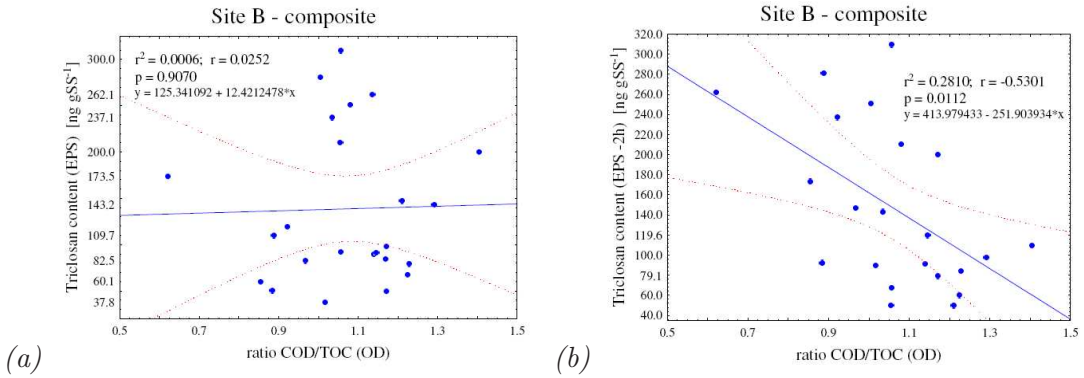


Figure 5.32: Correlation of COD/TOC *(specific oxygen demand*)* and Triclosan content EPS - Site B - 48-hour monitoring sampling , *(a)* COD/TOC (OD) vs Triclosan content EPS, *(b)* COD/TOC (OD) vs Triclosan content EPS after 2 hours (EPS-2h)

Assuming again a duration of two hours for the biomass to react to changes of wastewater properties of the bulk phase, one could conclude from Figure 5.31 and from Figure 5.32, that the Triclosan removal within the oxidation ditch might depend on the provision of substrates. This will be explained within the following paragraphs.

In Figure 5.31 *(b)* it can be seen that the higher the specific oxygen demand* of the bulk phase of the oxidation ditch is, the higher seems the Triclosan concentration which remains in the bulk phase after 2 hours (*r=0.5535*).

Whereas in Figure 5.32 *(b)* it is shown that, the lower the specific oxygen demand* is within the bulk phase of the oxidation ditch, the higher the uptake of Triclosan within the EPS gets after two hours (*r=0.5301*).

Therefore, it may be assumed in accordance to results found for COD results, that the biomass within the oxidation ditch seems to use Triclosan as additional substrate. However it should be noted again that the measured organic carbon represents the colloidal organic carbon fraction within the samples and that analyses could not be undertaken in duplicates, which might lead to the risk of high deviations from real values.

5.3.5 Soluble Proteins and Carbohydrates

Proteins and Carbohydrates are important parameters for residue contaminations in wastewater treatment plant discharges. They are an essential part of the dissolved biomolecules in wastewater and play a significant role as carbon source for heterotrophic bacteria in wastewater (Gremm and Kaplan, 1997). On the other hand they contribute to the formation of humic matter. Both classes of compounds are non toxic themselves nor *per se* persistent, although they are capable, like humic matter, to form complexes with metals (Abbt-Braun and Frimmel, 1991) or toxic substances (Jorand *et al.*, 1998) demasking therefore environmentally relevant substances. With changing pH-values or temperatures in the aquatic media it might be possible that those demasked substances can be released (Tsezos and Bell, 1991). They can also form complexes with bioactive substances such as endocrine disruptors or xenobiotica, which are environmentally harmful in low concentrations. Therefore proteins and carbohydrates might be a sink of pollutants in discharges of wastewater treatment plants (Huber, 1999). Extracellular polymeric substances play an important role in the formation and constitution of proteins and carbohydrates in wastewater effluents. This specific subject will be explained further in *Section 5.3.6 (Extracellular Proteins and Carbohydrates)*.

Proteins and carbohydrates in effluents of wastewater treatment plants can either be derived from the influents or are a product of biomass activity produced in the biological treatment stage. Proteins and carbohydrates entering the WTP with the influent can be eliminated within the biological stage by mineralisation and conversion (into inert intermediate products, into biomass respectively) or they may pass unchanged through the wastewater treatment. Huber (1999) revealed that up to 25% of the DOC in the effluents of municipal wastewater were shown to be biopolymers, such as proteins and carbohydrates.

Proteins are a group of compounds of natural macromolecule organic substances essential for anabolism and nutrition of all higher organisms. They consist of chains of polypeptides, which are formed by various amino acids.

Proteins *(soluble and extracellular)* were determined using the modified Micro Lowry procedure by Peterson (Peterson, 1977; Lowry *et al.*, 1951). The Peterson's Modification was chosen to eliminate the highly deviating results due to interfering compounds, possibly such as humic acids (McKinley and Vestal, 1985). Humic acids in wastewater were found to have high negative effect on the results achieved using the Bradford Method (Bradford, 1976) and Micro Lowry Onishi&Barr Modification (Peterson, 1977; Ohnishi and Barr, 1978). These studies have been conducted prior this study with artificial fed biomass and on a pilot scale RBC with real sewage feed *(unpublished data)*. The determination methods by Bradford and Micro Lowry Onishi&Barr Modification can be found in *Appendix 4.3.3.3.* Results obtained using the Peterson's Modification gave reliable deviations for wastewater samples.

The calibration curve produced from the suggested standards of the supplier yielded a plot with an obvious deviation of more than 5% of the Lambert-Beer law. This indicates interfering adsorption at high values and may be due to the use of disposable plastic cuvetes (Skoog *et al.*, 2000). According to Fisher Scientific the use of polystyrene plastic cuvetes result in a transmission of 85% at a wavelength between 500 - 600 nm, where protein determination was conducted. To reduce these effects calibration standards and samples were diluted in order to have an adsorption response of under 1.0. The plot of the calibration curve can be found in *Appendix 4.3.3.3.*

Carbohydrates are a widespread natural class of compound, to which mono-saccharides, their derivatives, and deduced di-, polygo- and polysaccharides all belong.

Determination of Carbohydrates *(soluble and extracellular - see section 5.3.6)* have been conducted using the method based on Dubois *et al.* (1956), however, as yielding in some cases resulted in very high standard deviations, this has not been found to be very reliable for such complex matrices as wastewater samples. The detection method by Dubois *et al.* (1956) is based on phenol-sulphuric reaction (5% phenol solution). Phenols are to be expected to be present in various concentrations in wastewater (Dignac *et al.*, 2000) and therefore might interfere the photometrical determination of carbohydrates (Huber, 1999). Besides Triclosan, which can be regarded itself as a phenolic compound, no other phenols or phenolic compound have been the subject of an investigation. The sometimes very high standard deviation of carbohydrates did not show any correlation with the detected concentrations of Triclosan. However, this may not provide any evidence if there would not be any other interference by other phenols or other wastewater contents. Dignac *et al.*

(2000), for instance, detected 0.3 mgL^{-1} soluble phenols in conventional wastewater. A total concentration of phenolic compounds similar to that would, in fact, be able to interfere with the phenol-sulfuric reaction. Furthermore, nitrite and nitrate are found to have infering effects on the determination method. However, Carbohydrate determination has been undertaken in triplicate and statistical analyses have been calculated by using the mean values. The calibration curve showed similar deviation to the Lambert-Beer law as with the Protein calibration and therefore may be an obvious influence of the used disposable cuvetes. Samples were therefore also diluted in order to yield an adsorbance below 1.0. The plot of the calibration curve can be found in *Appendix 4.3.3.4*.

5.3.5.1 Grab Samples

Proteins and Carbohydrates varied widely within the samples taken deriving from diurnal changes in wastewater composition. The average value in the effluents did not exceed, except for Site C, 10 mgL^{-1} for Proteins, 5 mgL^{-1} for carbohydrates, respectively (Figure 5.33). The higher average values for proteins and carbohydrates in the effluent of Site C might be influenced by the fact that discharging wastewater had to pass the lagoon, where possible release might take place. It should also be noted that the surface of this lagoon has always been covered with weed, which might lead to higher bioactivity and therefore possible impacts on the concentration of proteins and carbohydrates within the liquid phase.

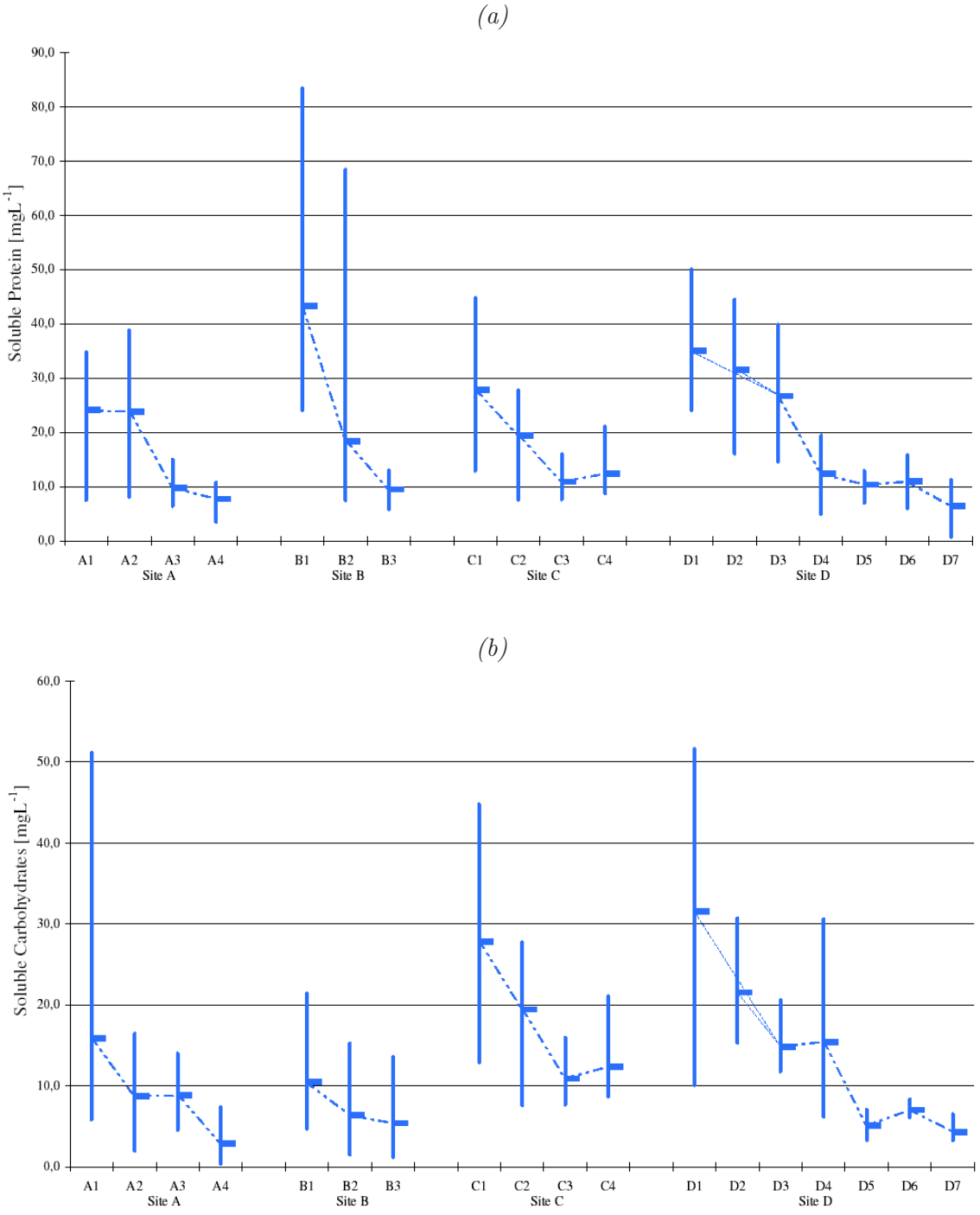


Figure 5.33: Variation of *(a)* Soluble Proteins [mgL^{-1}] and *(b)* Soluble Carbohydrates [mgL^{-1}] - Site A, B, C, D

Triclosan removal rates of **Site A** showed for the crude influent (*A1*) and the RBC-effluent (*A3*) significant increase with the increasing removal rate of **Proteins** (*r=0.9214, p=0.0096*) and a slight correlation with the ratio of **Proteins/Carbohydrates** removal (*r=0.6435, p=0.1680*), whereas no relevant correlations could be observed for carbohydrates, but it must be noted that the carbohydrate removal correlated with the protein removal. The relevant Triclosan graphs can be seen in Figure 5.34.

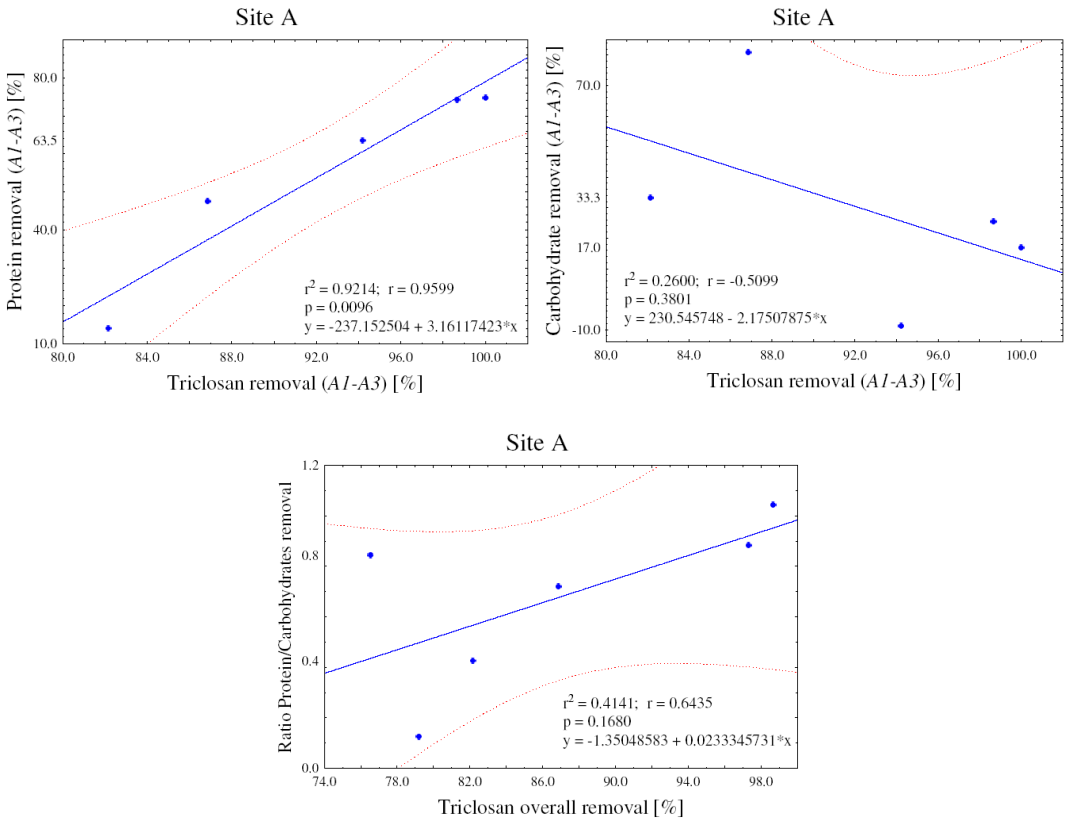


Figure 5.34: Correlation of Proteins and Carbohydrates - Site A

Site B showed no correlations at all for either **proteins** or **carbohydrate** removal rates or **Proteins/Carbohydrates** ratio. Figure 5.35 shows the statical analysed graphs.

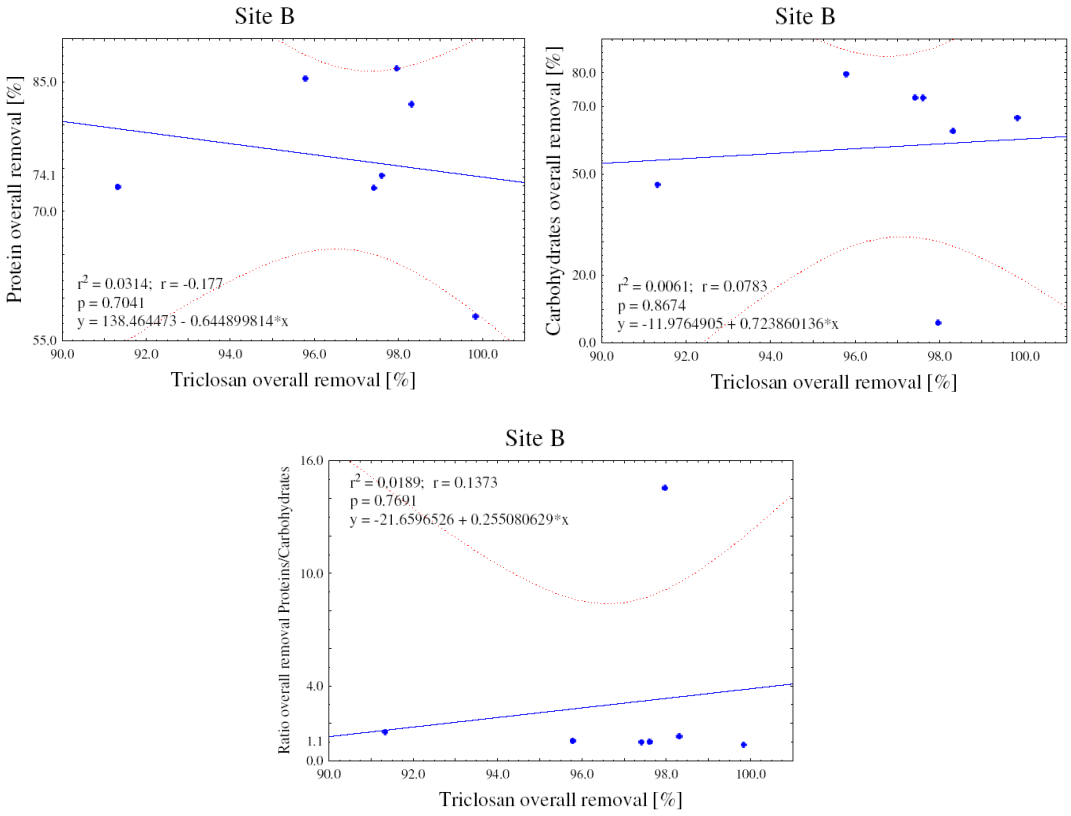


Figure 5.35: Correlation of Proteins and Carbohydrates - Site B

As the concentrations of proteins and carbohydrate at the discharging effluent for **Site C** correlation graphs have also been plotted for values of the Influent (*C1*) and Humus Effluent (*C3*). In contrary to WTP A, **proteins** seem to have no significant influence (*r=0.0942, p=0.8803*), whereas carbohydrates seemed to have some influence (*r=0.8682, p=0.0563*). **Proteins/Carbohydrates** removal showed no relevance for both overall removal and removal between C1-C3. Correlation graphs are shown in Figure 5.36.

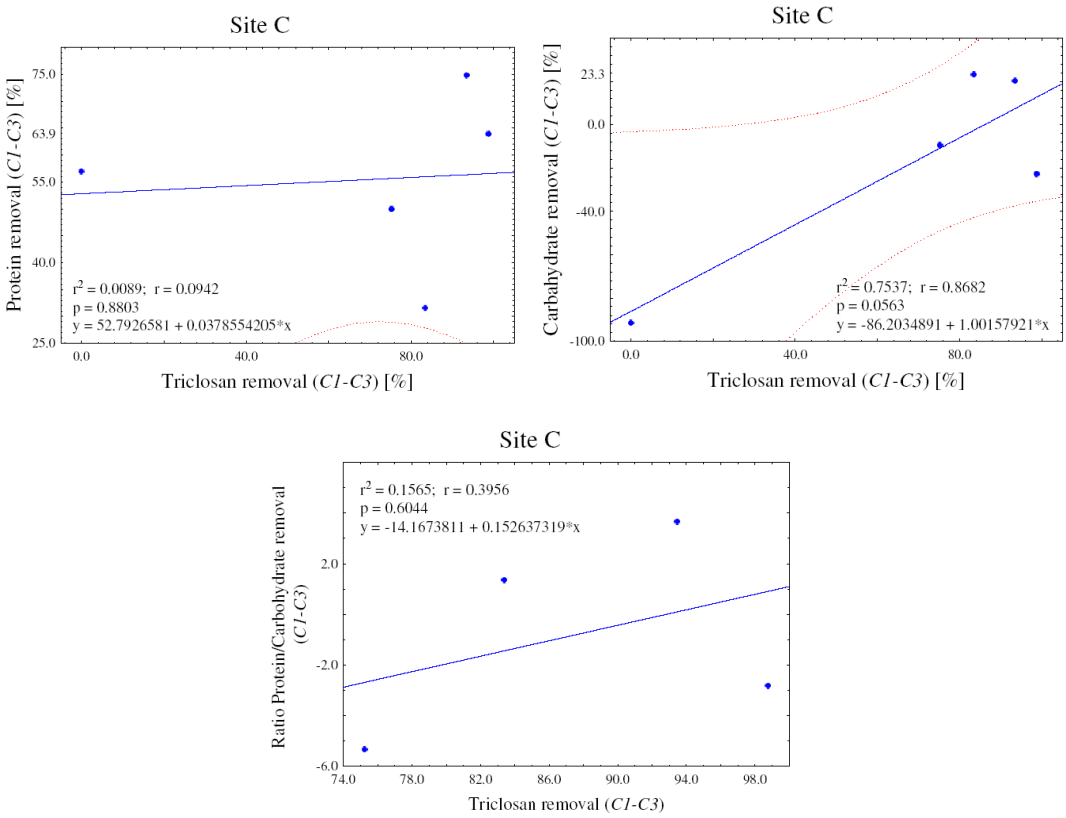


Figure 5.36: Correlation of Proteins and Carbohydrates - Site C

Site D showed no correlation for **proteins** or **carbohydrates** removal rates, but did however for the ratio of **Proteins/Carbohydrates** overall removal (*r=0.9574, p=0.0426*). Graphs are shown in Figure 5.37.

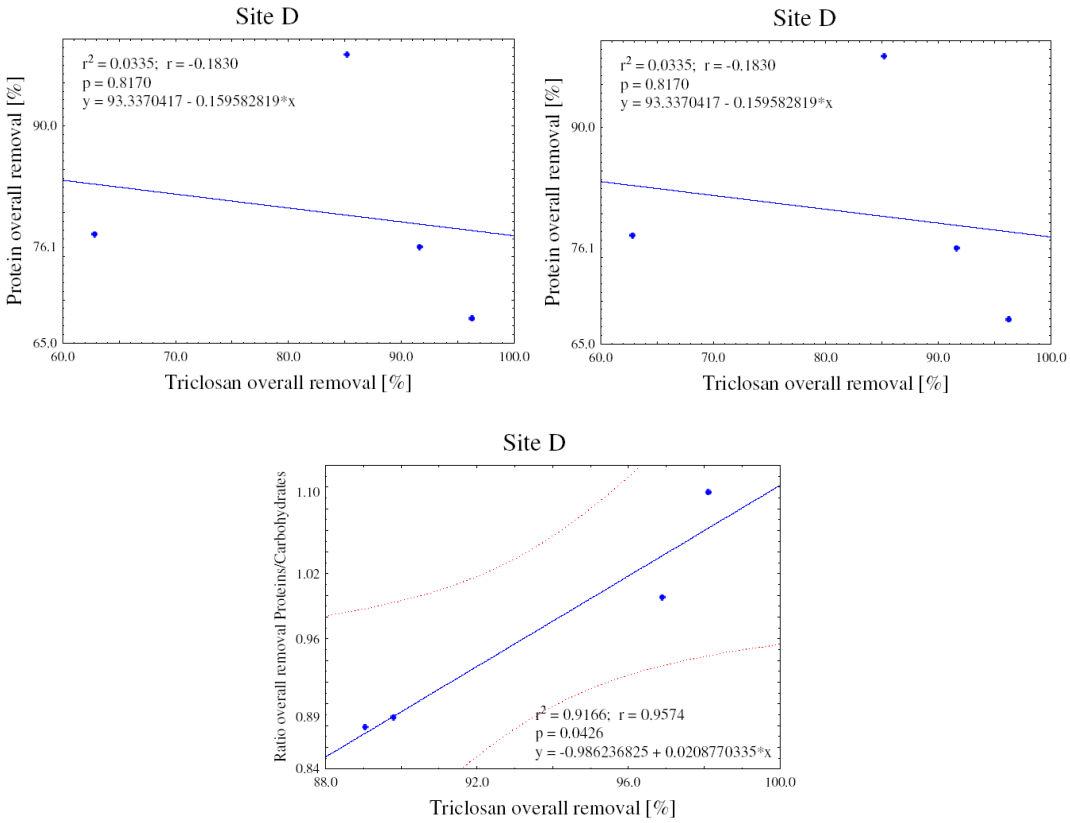
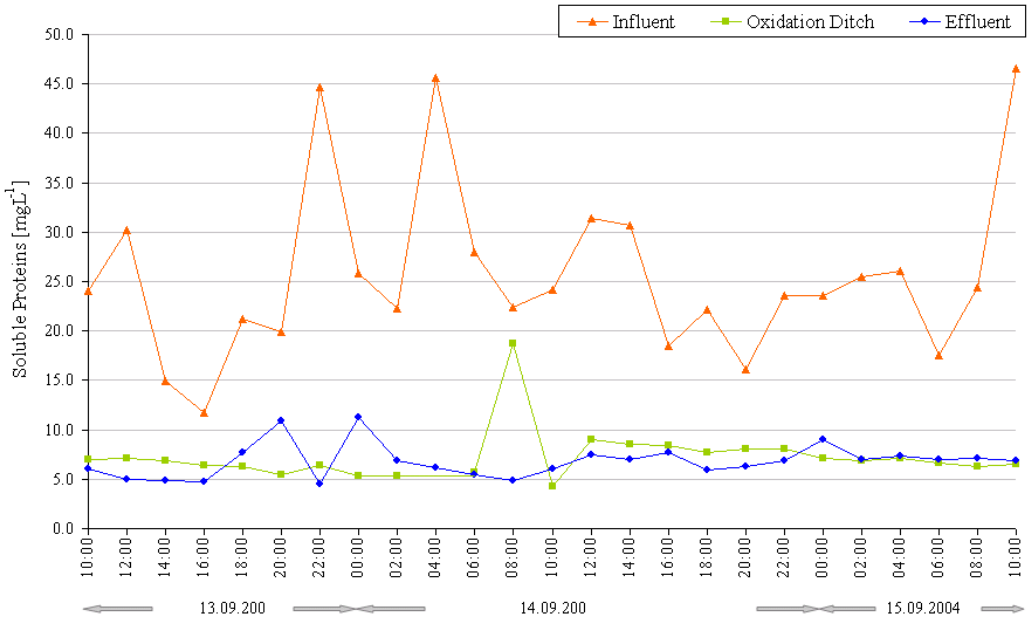


Figure 5.37: Correlation of Proteins and Carbohydrates - Site D

5.3.5.2 48-hour monitoring sampling

Soluble proteins and carbohydrates varied widely between samples as expected. The carbohydrate values often showed wide standard deviations of replicates, revealing once again that the method used by Dubois *et al.* (1956) was not entirely reliable for measuring such complex matrices as wastewater. The variations in samples are given in Figure 5.38.

(a)



(b)

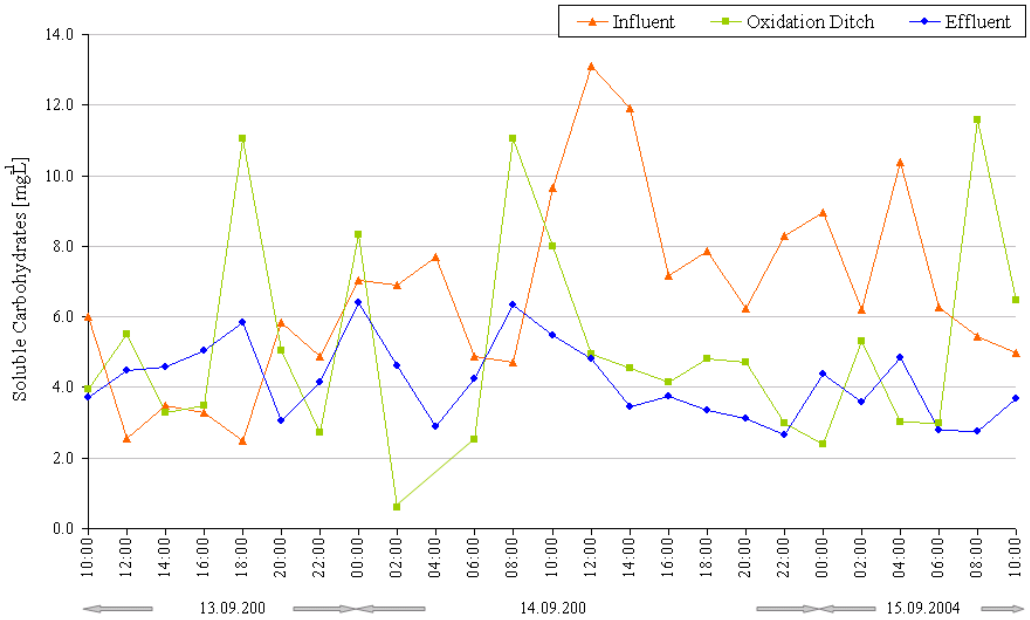
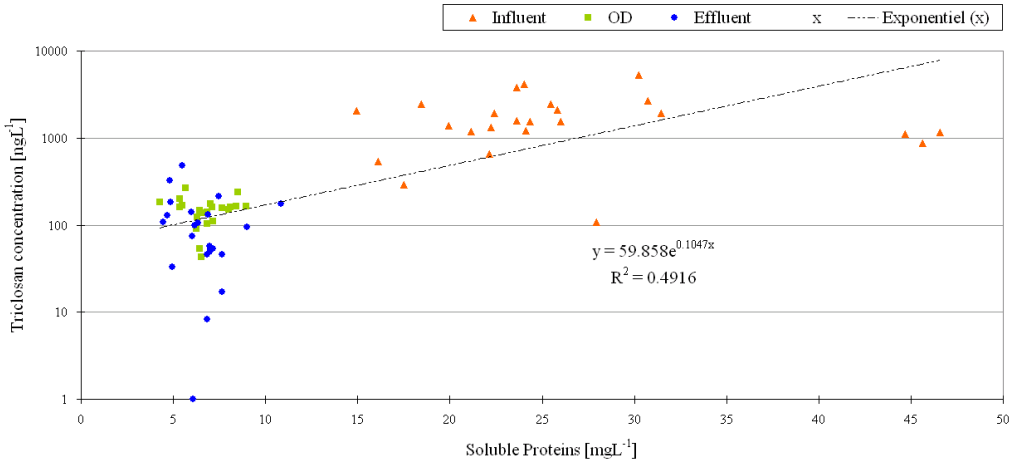


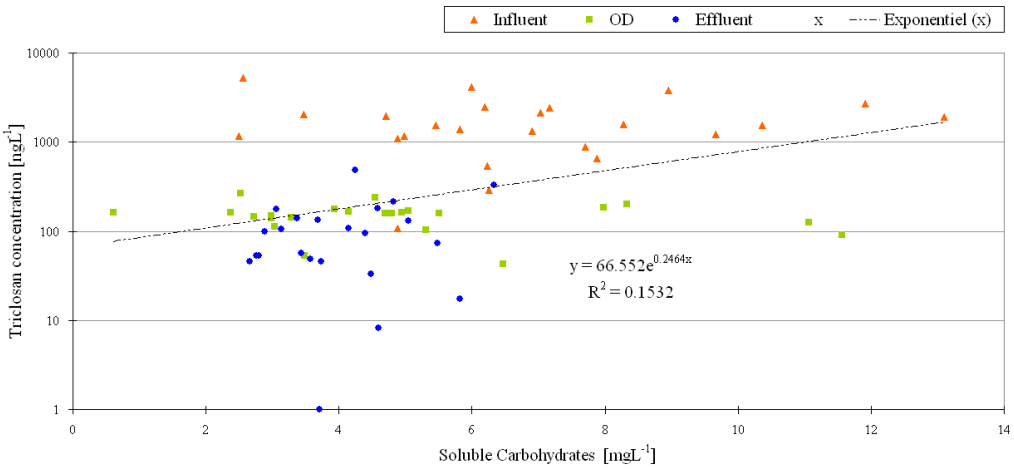
Figure 5.38: Variation of *(a)* Soluble Proteins [mgL^{-1}] and *(b)* Soluble Carbohydrates [mgL^{-1}] - Site B - 48-hour monitoring

Plotting concentrations of Proteins, Carbohydrates respectively, against Triclosan concentrations resulted in correlations of R^2=*0.4916* for Proteins, R^2=*0.1532* for Carbohydrates respectively *(see Figure 5.39)*.

However, analogues COD and TOC values this analysis does not allow any conclusion about the correlations between the parameters within each treatment stage. Correlations for Triclosan concentration to Protein concentrations and to Carbohydrate concentrations respectively, for Influent(*Proteins: r=-0.0263, Carbohydrates: r=-0.0365*), Effluent (*Proteins: r=-0.0297, Carbohydrates: r=0.2903*) and Oxidation Ditch (*Proteins: r=0.0825, Carbohydrates: r=-0.1504*) can be seen in Figure 5.40



(a)



(b)

Figure 5.39: *(a)* Soluble Protein and *(b)* Soluble Carbohydrate concentration vs Triclosan concentration - Site B - 48 hour monitoring

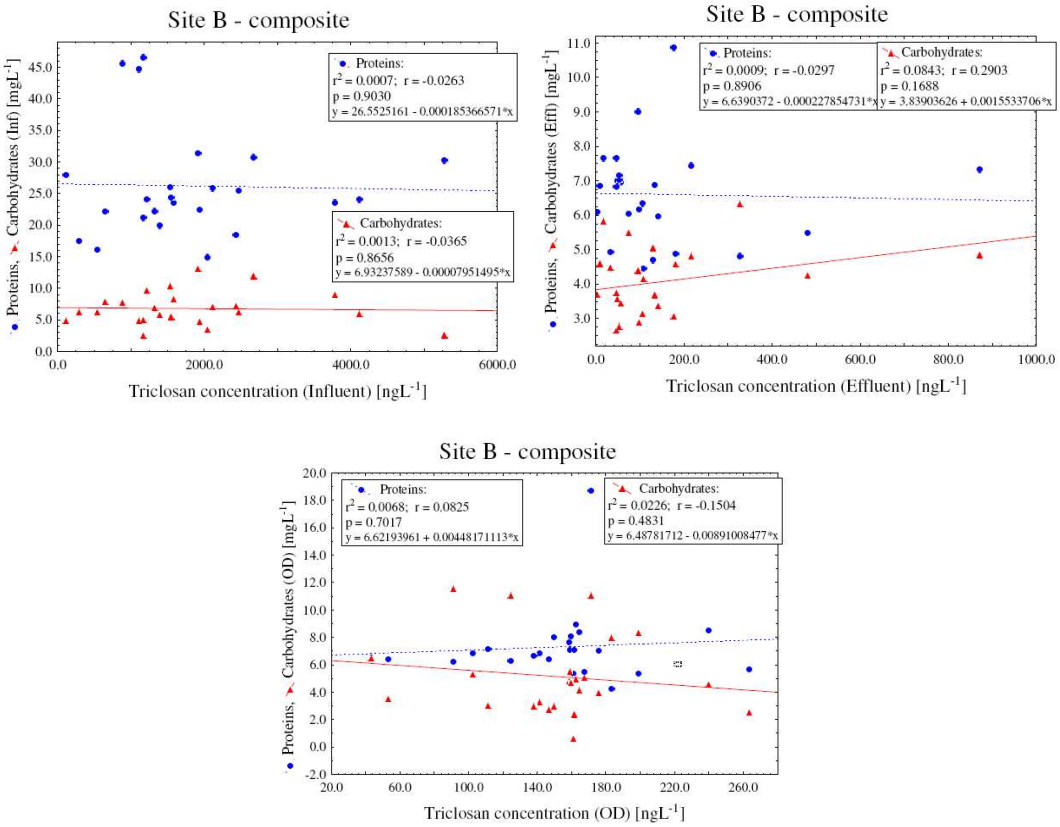


Figure 5.40: Correlation of Protein, Carbohydrate concentrations and Triclosan concentrations for Influent, Effluent, OD - Site B - 48-hour monitoring sampling

Figure 5.40 shows that there are no correlations between the actual concentrations of Proteins, Carbohydrates respectively, and Triclosan concentration within the bulk phase of each treatment stage. Additionally, similar to the grab sampling, the Triclosan overall removal within the 48-hour monitoring showed no correlations at all for either proteins (*r=0.0308*) and carbohydrate removal rates, (*r=-0.1977*) or removal rates of Proteins/Carbohydrates ratio (*r=0.9780*). Figure 5.41 gives the statistical analysed graphs.

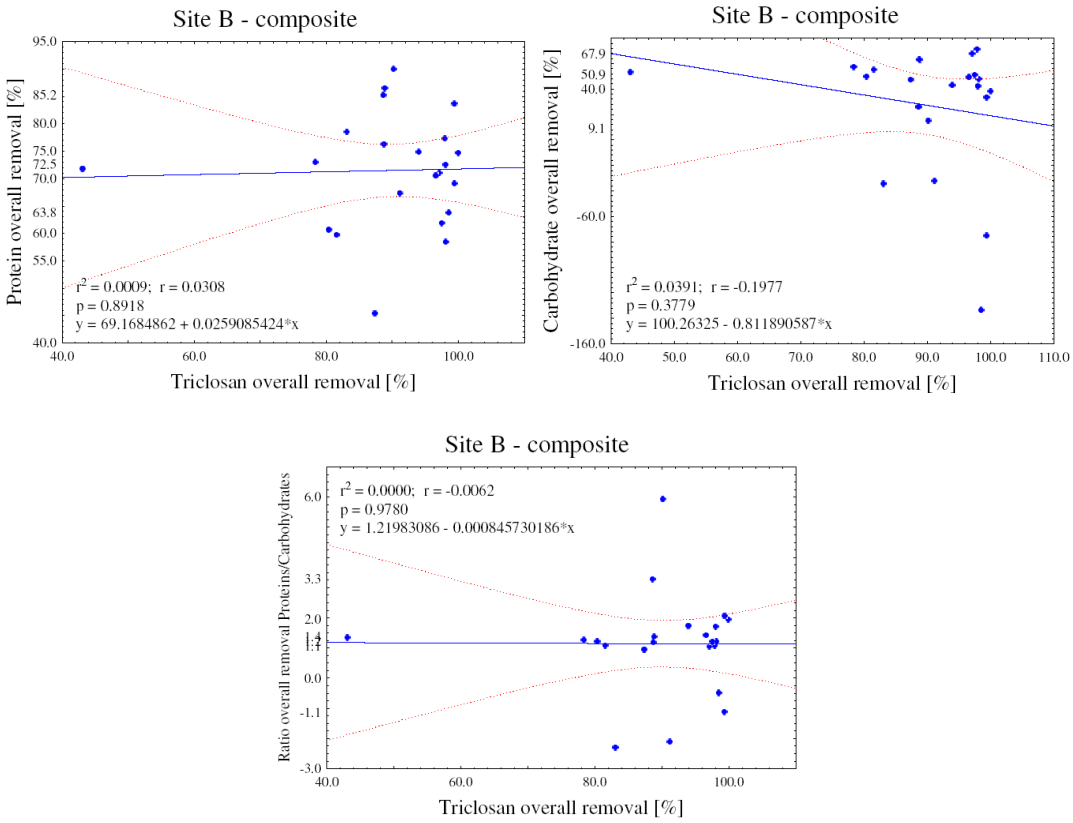


Figure 5.41: Correlation of Proteins and Carbohydrates - Site B - 48 hour monitoring

However, the primary removal rate of Triclosan (removal Influent-Oxidation) seemed to be connected to soluble protein and carbohydrate concentrations within the oxidation ditch after two hours. Figure 5.42 shows the statistical data graphs, where it can be seen that the higher the concentration of proteins, carbohydrates respectively, within the bulk phase of the oxidation ditch was, the lower the Triclosan primary removal seemed to get. Correlations for proteins (*r=-0.8702*) were found to be higher than those for carbohydrates (*r=-0.5458*), which is, in fact, due to one outstanding data set of negative Triclosan removal to very high soluble protein concentrations within the oxidation ditch.

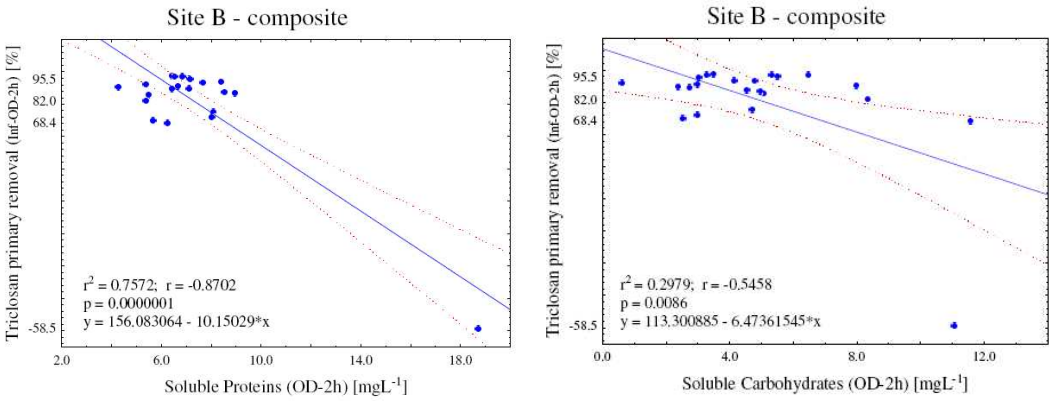


Figure 5.42: Correlation of Soluble Proteins and Carbohydrates within the Oxidation Ditch (OD-2h) to Triclosan Primary Removal (Inf-OD)

This observation might again be suggested to higher Triclosan removal, whenever there is a shortage of available substrates. This may be concluded as well from the following Figure, Figure 5.43, where concentrations of Proteins, Carbohydrates respectively, showed moderate correlations to Triclosan concentrations of the bulk phase within the oxidation ditch after two hours (*Proteins (Inf): r=0.6292*, *Carbohydrates (Inf): r=0.3356*). Assuming again two hours reaction time of biomass to changes of the bulk phase, one could conclude that the Triclosan concentration which remained within the bulk phase of the oxidation ditch seemed to depend on the provision of substrates provided by the receiving wastewater (influent). Hence, the higher the Protein concentrations within the influent was, the higher the Triclosan remained within the bulk phase of the oxidation ditch (*r=0.6292*). The concentrations of Carbohydrates in the influent seemed to be less important (*r=0.3356*), whereas the ratio of Proteins to Carbohydrates revealed in no numerical correlation (*P/C(Inf): r=0.1491*, see also Figure 5.43).

No correlation could be observed for concentrations of Proteins within the bulk phase of the oxidation ditch and the remaining Triclosan concentration within the bulk phase of the oxidation ditch after two hours, neither was there any numerical correlation for protein and carbohydrate concentrations and Triclosan values within the effluent (*graphs not given*).

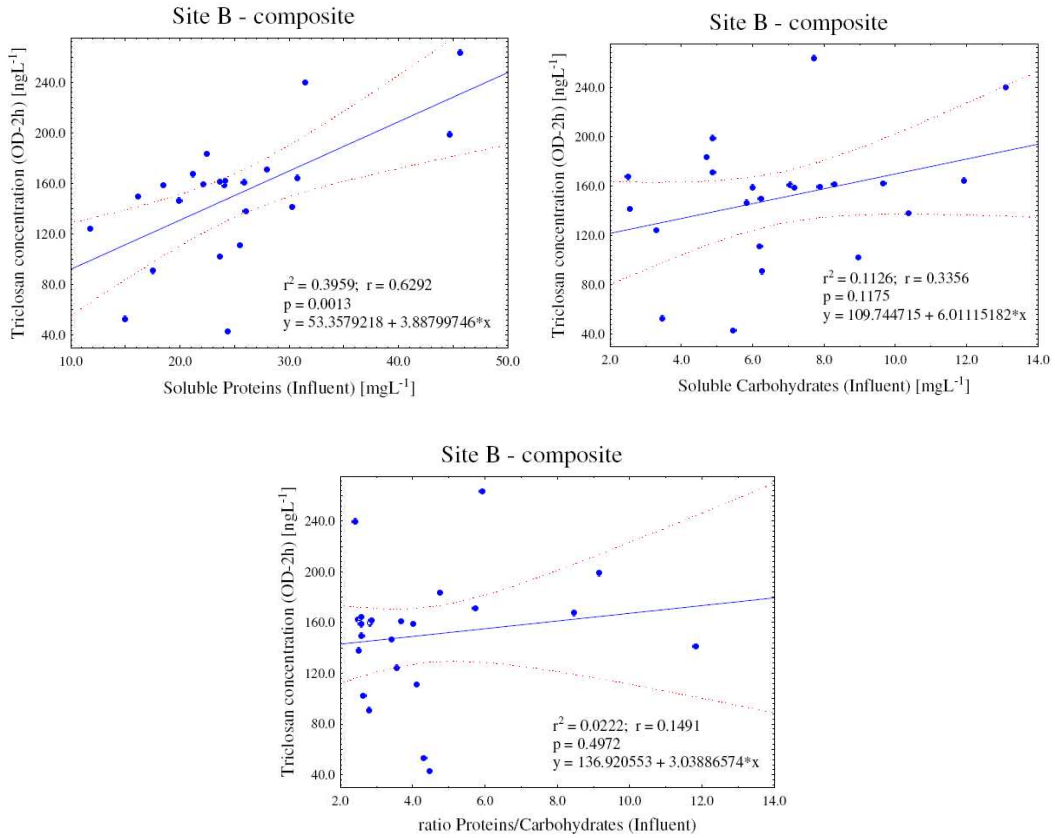


Figure 5.43: Correlation of Proteins and Carbohydrates (Influent) vs Triclosan concentration within the bulk phase of the oxidation ditch after two hours (OD-2h)

Summarising it can be said, that soluble proteins and carbohydrates concentrations varied widely for each sampling location. They only seemed to have an occasional influence on overall Triclosan removal within the grab sampling period, which might be due to the sampling stratification. Soluble proteins and carbohydrates concentration for the 48-hour monitoring lead to the assumption that Triclosan removal within the oxidation ditch might be influenced by available substrates from the inflowing wastewater.

However, it should be noted that Protein and Carbohydrate determination resulted occasionally in high standard deviations and therefore, found correlations might be suspect to be influenced by the high deviations.

It must also be noted that these processes are very complex and cannot be regarded in such a simple manner. There are many possible influences on the behaviour of EPS, soluble proteins and carbohydrates and the compound of interest, Triclosan, which may not have been investigated during this study, such as dissolved oxygen, salinity or other available nutrients.

5.3.6 Extracellular polymeric substances

Biofilms are an accumulation of microorganisms (prokaryotic and eukarotic unicellular organisms), extracellular polymeric substances (EPS), multivalent cations, biogenic and inorganic particles as well as colloidal and dissolved compounds (Wingender *et al.*, 1999). **Activated Sludge flocs** are thought to consist of microbial aggregates, filamentous organisms, extracellular polymeric substances, organic and inorganic particles (Bruss *et al.*, 1992). The common feature of all these aggregates is that the microorganisms are embedded in a matrix of **extracellular polymeric substances (EPS)** (Wingender *et al.*, 1999).

Extracellular polymeric substances appear as a highly hydrated capsule attached to the cell or as a viscous, soluble slime (Christensen and Characklis, 1990). EPS are thought to act as a cushion layer for microorganisms within biofilms or activated sludge flocs. They mainly consist of polysaccharides (carbohydrates), proteins, DNA and lipids (Goodwin and Foster, 1985). Besides water and cell content, extracellular polymers make up the third biggest part within activated sludge flocs (Li and Ganczarczyk, 1990) and their concentration within biofilms is according to Christensen and Characklis (1990) between 1-2 % (w/w). The extent and composition of these polymers vary with physiological state of the organisms and the external conditions (Characklis and Marshall, 1990; Schmitt *et al.*, 1995). The chemical composition and the properties of EPS are responsible for the cohesion and the mechanical stability of biofilms/activated sludge flocs. Extracellular polymers contain hydrophobic substances, such as proteins and lipids, where van der Waals forces can act between them (Urbain *et al.*, 1993). In addition, hydrophobic interactions can occur between proteins and lipids of the EPS and the hydrophobic groups of the cell surface, which increase the bond of EPS to the cells. Due to high content of anionic groups, such as phosphates, carboxylates or sulfates, electrostatic interactions occur between the different biopolymers. The stability of biofilms and activated sludge flocs can be increased by the prevalence of divalent cations, such as calcium or magnesium, bridging the chains of polysaccharides (Bruss *et al.*, 1992).

The properties of the EPS may play a critical role in understanding the physical and physiological behaviour of biofilms/activated sludge flocs. EPS are considered to play an important role in dewatering of activated sludge (Urbain *et al.*, 1993; Neyens *et al.*, 2004) and in the removal of pollutants from wastewater in bioflocculation and settling (Eriksson and Alm, 1991; Bruss *et al.*, 1992; Urbain *et al.*, 1993; Liu *et al.*, 2001). In general, fixed biofilms are known to have a higher resistance towards toxic shock loads in comparison to suspended solids. This suggests that the higher toxic resistance is based on diffusion barriers within the biofilm matrix exopolymers (Wuertz *et al.*, 1998). Sorption processes can enhance the biodegradation by removing inhibitory compounds from solution, thereby reducing their toxic effects on microbial growth (Bouwer, 1989). Thus attention must be

paid to the interaction between cell physiology, EPS properties, and the resulting effect on bulk properties. It is necessary to characterise these polymers as, in many cases, EPS are used to explain otherwise unexplainable phenomena (Christensen and Characklis, 1990).

EPS within wastewater treatment works vary hourly depending on the properties of the influent (Bouwer, 1989; Späth *et al.*, 1998). According to the extraction method, which is not yet standardised, the composition of EPS may also vary widely. Morgan *et al.* (1990) found a ratio of Proteins/Carbohydrates of 0.8 for activated sludge, whereas the ratio of Proteins/Carbohydrates for activated sludge found by Frølund *et al.* (1996) yielded from 3.9 - 5.1 being depending on the duration of the extraction time.

Within this study, extraction of EPS has been conducted by a Cranfield University standard method, based on a modified heating method by Zhang *et al.* (1999). As this method has been used in majority for suspended solid growth systems, it had to be modified slightly in order to be applicable to fixed biofilms (RBC-sludge samples (*A5*)). Due to the fact that no cell lysis studies could be undertaken, it might well be that the results obtained are contaminated by intracellular substances yielding in higher EPS values. Results for extracellular substances might have also been influenced by the usage of glass filter paper with a pore size diameter $\gg 0.45$ μm, where filtrated samples could be contaminated with cells and cell lysis products. However, aliquots of supernatants (soluble and extracellular) filtrated once through a 0.45 μm Wattmann membrane filter (Fisher Scientific) and analysed for proteins, carbohydrates and TOC, did not show significant differences in results (*<0.05 - 0.3%*). They were found to be negligible regarding the much higher average standard deviation of, for instance, carbohydrate determination. Control of aliquots results ought to be done as well with a pore size diameter of 0.22 μm, but has not been possible for this study.

5.3.6.1 Extracellular Proteins and Carbohydrates - Grab Samples

Figure 5.44 gives an overview of extracellular Proteins (P_{EPS}) and extracellular Carbohydrates (C_{EPS}) normalised to volatile suspended solids, volatile total solids, respectively. Horizontal bars represents the variation of minimum to maximum values, whereas vertical small bars represents the average values for all sampling occasions. The ratio between P_{EPS}/C_{EPS} are given in Table 5.7.

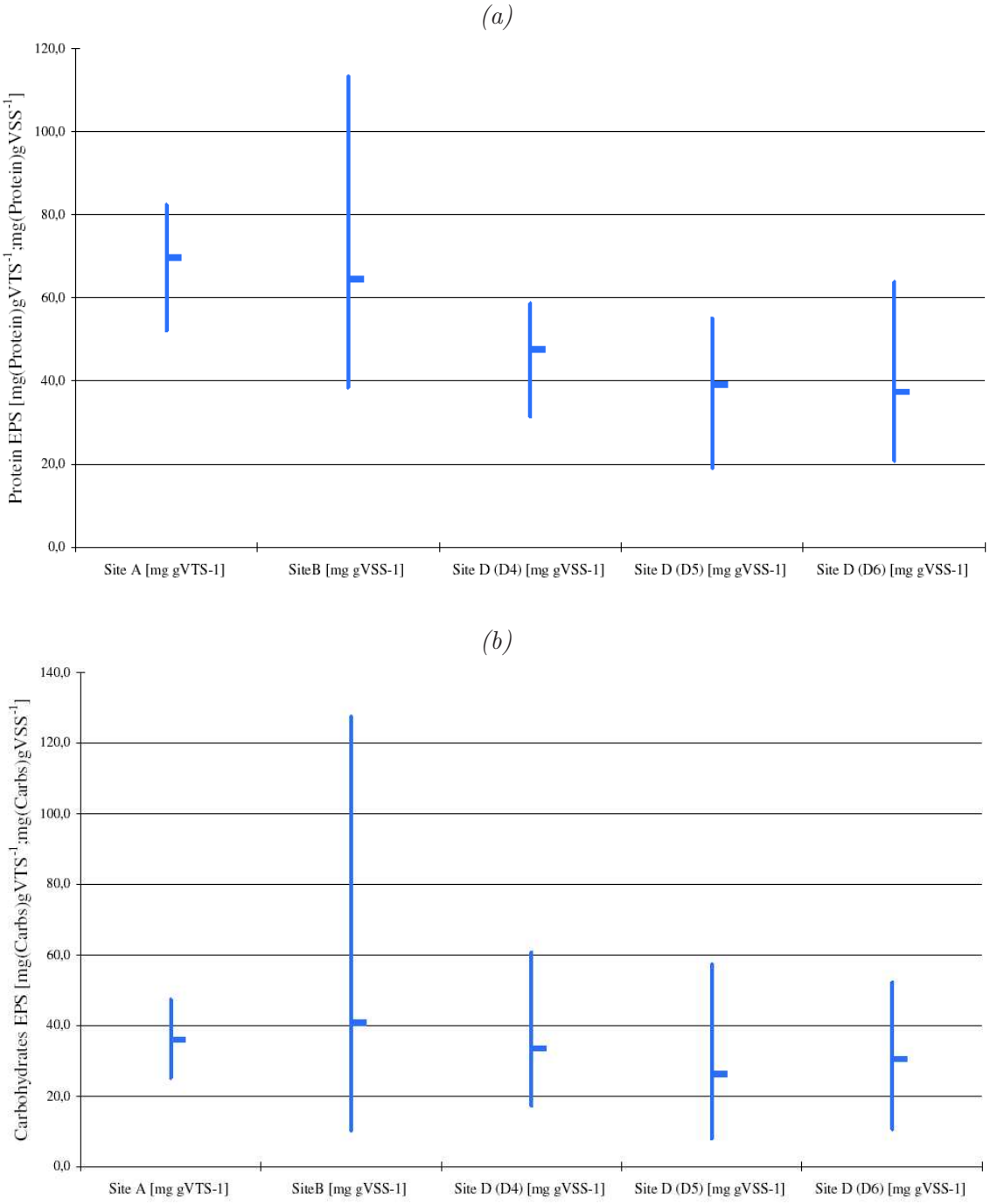


Figure 5.44: Variation of *(a)* Extracellular Proteins and *(b)* Extracellular Carbohydrates - Site A, B, C, D

Table 5.7: Ratio P_{EPS}/C_{EPS} - Site A,B,D

Site	type of sludge sample	range of ratio P_{EPS}/C_{EPS} (median)
A	A5 - fixed biofilm (RBC)	1.4 - 3.2 (2.1)
B	B2 - suspended solids (oxidation ditch)	0.5 - 4.1 (2.6)
D	D4 - suspended solids (anoxic lane)	1.0 - 2.3 (1.7)
	D5 - suspended solids (aerob lane)	1.0 - 3.3 (2.2)
	D6 - suspended solids (RAS)	0.5 - 2.2 (1.6)

The concentration of extracellular proteins varied from 20 to 115 mg $gVSS^{-1}$ for suspended solid systems and from 50 to 85 mg g VTS^{-1} for fixed biofilms. Concentrations of extracellular carbohydrates are in general lower, ranging from 10 to 60 mg $gVSS^{-1}$, mg VTS^{-1}, respectively, except for the oxidation ditch sample (*B2*) where extracellular carbohydrate concentration exceeded 125 mg $gVSS^{-1}$ on one occasion. However, the average concentration for C_{EPS} is approximately 40 mg $gVSS^{-1}$. The ratio of P_{EPS}/C_{EPS} are between 0.5 and 4.1. On average the ratio of P_{EPS}/C_{EPS} varies between 1.6 and 2.6, demonstration that proteins are in general the more prevalent extracellular compounds. Ratio values are comparable to other literature data, where either protein or carbohydrate concentration dominated the extracellular composition depending on the extraction time. Liu *et al.* (2001) found a ratio of P_{EPS}/C_{EPS} of 3.5 (heating method, 80°C, 30 min) and Morgan *et al.* (1990) a ratio of 0.8 (heating method, 80°C, 10 min) for activated sludge samples. An overview of Literature data is given in Table 5.8.

Table 5.8: Literature data of EPS

P_{EPS}[mg $gVSS^{-1}$]	C_{EPS}[mg g VSS^{-1}	ratio P_{EPS}/C_{EPS}	type of sludge	extraction method	references
44.9	13.0	3.5	activated sludge	heating (30min, 80°C)	Liu *et al.* (2001)
16.7	9.9	0.8	activated sludge	heating (10min, 80°C)	Morgan *et al.* (1990)

Concentration of C_{EPS}, P_{EPS} or the ratio of P_{EPS}/C_{EPS} (*r=0.0595, p=0.9243*) showed no significant correlation to overall Triclosan removal for **Site A**, whereas the ratio of extracellular proteins (P_{EPS}) to soluble proteins (P_{SMP}) seemed to have important impact on the overall Triclosan removal (*P_{EPS}/P_{SMP}(A5), r=0.9596, p=0.0404*). The ratio of extracellular (C_{EPS}) to soluble carbohydrates (C_{SMP}) also showed some correlation (*p=0.8101*) but was outside the borderline of statistical significance (*p=0.1899*). STATISTICA graphs are given in Figure 5.45.

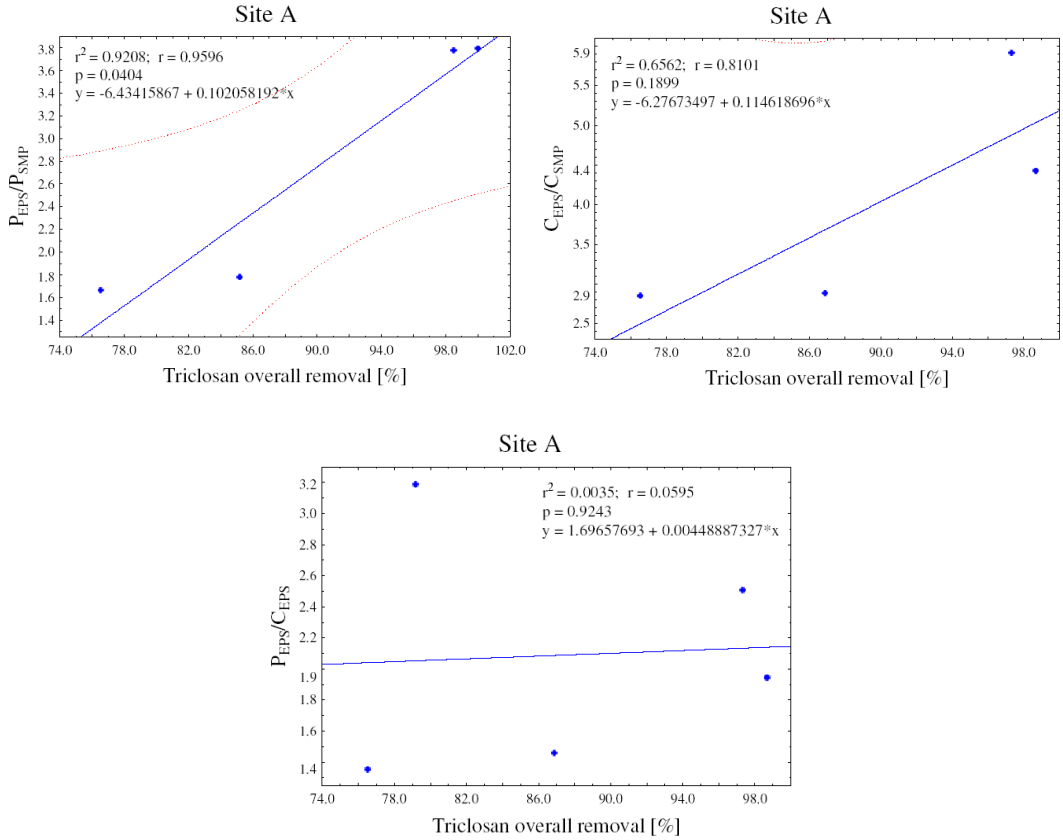


Figure 5.45: Correlation of extracellular Proteins and Carbohydrates - Site A

Site B showed in contrast to Site A, a decrease in overall Triclosan removal with increasing ratio of P_{EPS}/P_{SMP}(*r=-0.6338, p=0.1766*), though not significant. The ratio of carbohydrates C_{EPS}/C_{SMP} showed no correlation to overall Triclosan removal (*r=0.3211, p=0.5349*), neither did the ratio of P_{EPS}/C_{EPS} (*r=-0.0999, p=0.8507*). Graphs are given in Figure 5.46.

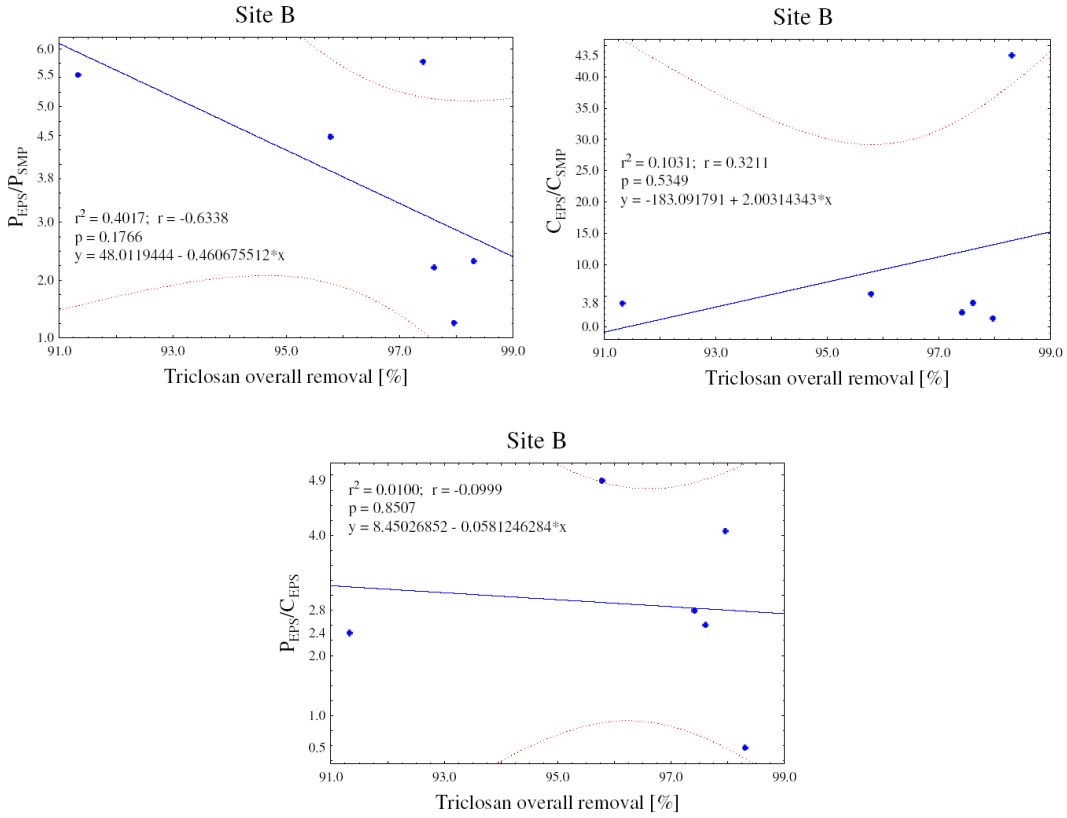


Figure 5.46: Correlation of extracellular Proteins and Carbohydrates - Site B

Three types of sludge; anaerobic (*D5*), anoxic (*D4*) and returned activated sludge (*D6*) were analysed from **Site D**. As this WTP has not been sampled as often as the three other sites A, B, C correlation was often only undertaken using three data pairs. This is, in fact, a very poor basis for statistical analysis. Therefore, only the apparently relevant correlation will be given as graphs in this section (Figure 5.47). In contrast to the observed EPS correlations of Site A and B, Site D did not show a significant correlation for P_{EPS}/P_{SMP} or C_{EPS}/C_{SMP} ratio for any type of sludge sample (*D4, D5, D6*), whereas the ratio of P_{EPS}/C_{EPS} seemed to increase with increasing overall removal of Triclosan (*D4: r=0.9988, p=0.0316; D5: r=0.9989, p=0.0294*). Though the ratio of P_{EPS}/ C_{EPS} correlated for the returned activated sludge (r = *0.9825*), it was not shown to be significant (*p=0.1192*). The correlation graphs can be seen in Figure 5.47.

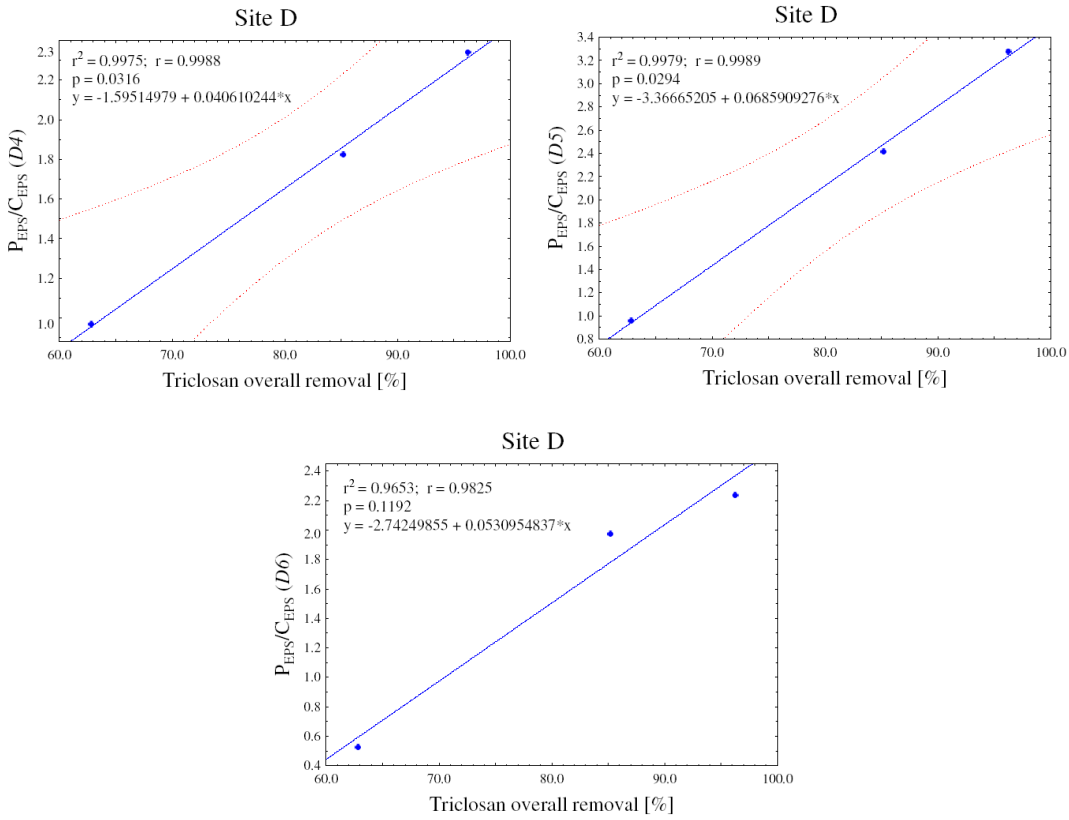


Figure 5.47: Correlation of P_{EPS} / C_{EPS} and Triclosan overall removal - Site D

No known record exists regarding the influences of extracellular composition on the removal of Triclosan. Even though extracellular protein and carbohydrate concentration seemed to correlate in some cases of the grab sampling period with overall Triclosan removal, no unequivocal impact could be figured out on the basis of this study. According to Liao *et al.* (2001), the ratio of protein to carbohydrate effectuates the hydrophobicity of the extracellular polymeric substances, providing more possible binding sites to hydrophobic compounds. Jorand *et al.* (1998) revealed that hydrophobic fraction in EPS is due to proteins, but not carbohydrates, whereas Morgan *et al.* (1990) found a correlation between surface charge of activated sludge flocs and the composition of EPS.

However, as this study could not be conducted within hydraulic flow correlating samples, the results obtained have to be handled with care. It should be noted that EPS may alter hourly (Späth *et al.*, 1998) and it should also be noted that there might be other, more complex influences on the ratio of proteins/carbohydrates or the overall Triclosan removal, such as fat content, temperature, nutrients or pH changes. Furthermore, the EPS extraction had to be undertaken for two sampling months (*May and June*) by micro-centrifugation, due to a technical failure of the Rotana 96 R centrifuge and therefore there is only a limited comparison possible.

5.3.6.2 Extracellular Proteins and Carbohydrates - 48-hour monitoring sampling

The EPS composition showed, as expected, wide diurnal variations. The average for P_{EPS} (55 mg g VSS^{-1}) and C_{EPS} (21 mg g VSS^{-1}) are slightly lower than those for the grab samples, Site B (P_{EPS}: *68 mg g* VSS^{-1}, C_{EPS}: *40 mg g* VSS^{-1}). The concentration of extracellular proteins and carbohydrates normalised to VSS are given in Figure 5.48.

The ratio of P_{EPS}/C_{EPS} varied between 1.7 and 3.4 (average 2.4) and is supplemented in Figure 5.49 with the level of the soluble microbial products, P_{SMP}/C_{SMP}.

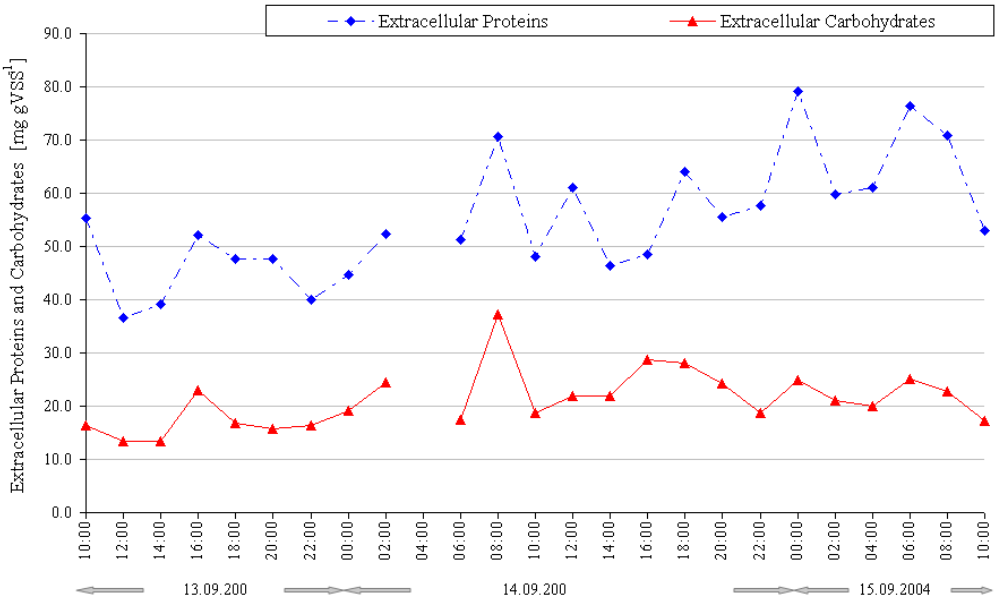


Figure 5.48: Variation of Extracellular Proteins [mg g^{-1}VSS] and Extracellular Carbohydrates [mg g^{-1}VSS] - Site B - 48 hour monitoring

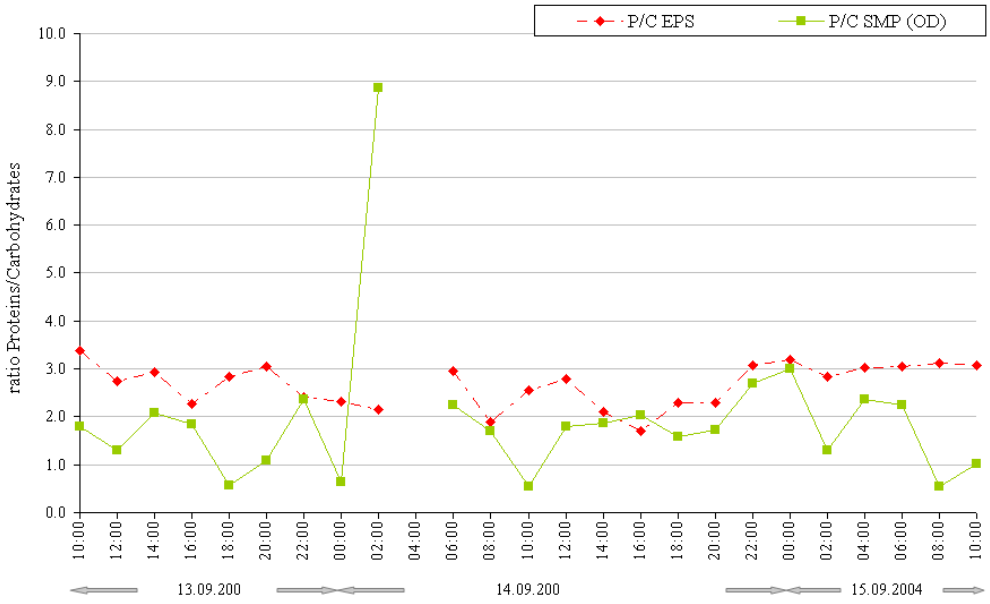


Figure 5.49: Comparison of ratio of Proteins/Carbohydrates in EPS and SMP (OD) - Site B - 48 hour monitoring

In general the level of SMP proteins and carbohydrates were below the level of EPS, except for one sampling point, where the very high ratio of P_{SMP}/C_{SMP} was due to very low soluble carbohydrate concentration. No correlation could be found to Triclosan overall removal rates (Figure 5.50). This is in accordance to the observation made during the grab sampling for Site B. Furthermore, this might suggest that the previously observed effects that occurred during the grab sampling period for Site A, D may have been random or only occur with different sludge types, which can not be specified further on the basis of this study. EPS concentration and therefore level of EPS/SMP, might be artificially increased due to possible contamination of intracellular materials (filter pore size $\gg 0.22$ μm, no cell lysis study) and, even if very low, due to the fact that sludge sample were not pre-washed prior EPS extraction.

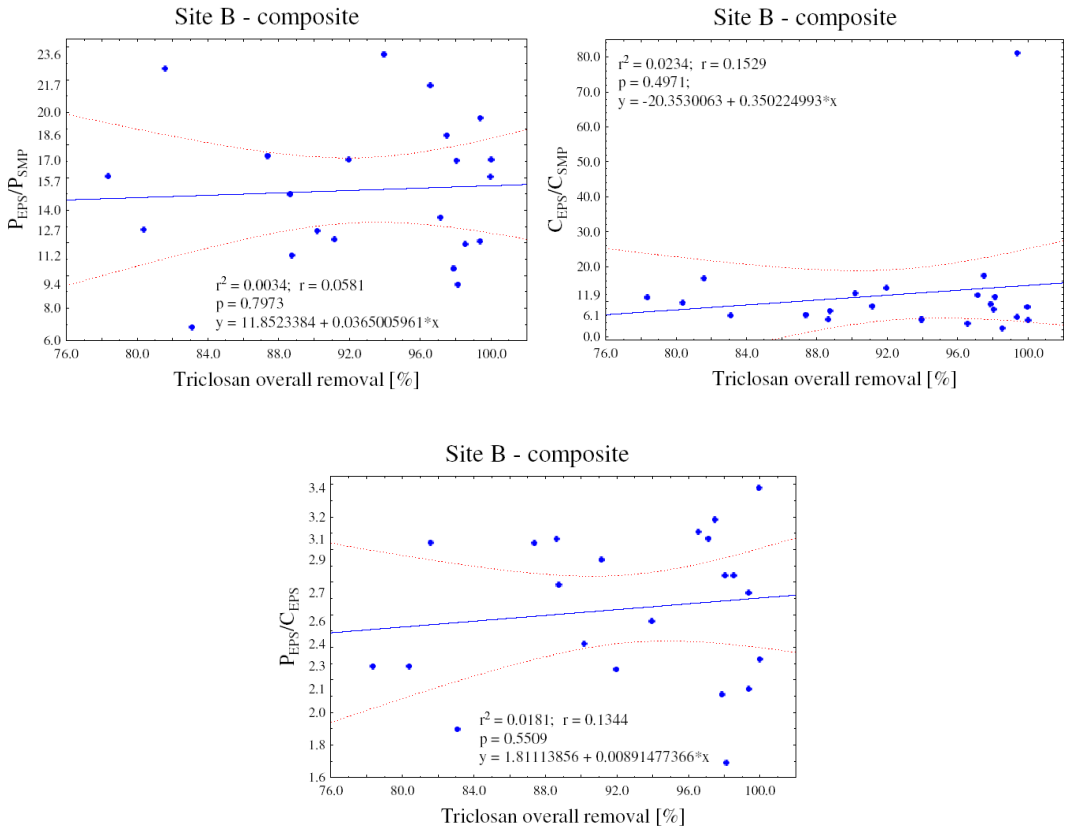


Figure 5.50: Correlation of P_{EPS}/C_{EPS} and Triclosan overall removal - Site B - 48 hour monitoring

However within this study the Triclosan concentration of the bulk phase of the oxidation ditch showed to correlate to concentrations of extracellular carbohydrates after two hours (*r=0.5883*) and to the ratio of P_{EPS}/C_{EPS} after two hours (*r=-0.6803*), but not to extracellular proteins after two hours (*r=0.0362*).

Statistical graphs are given in Figure 5.51 and it can be seen that the rising Triclosan concentration within the bulk phase of the OD seemed to cause a higher carbohydrate content within the EPS, resulting in a lower ratio of P_{EPS}/C_{EPS}.

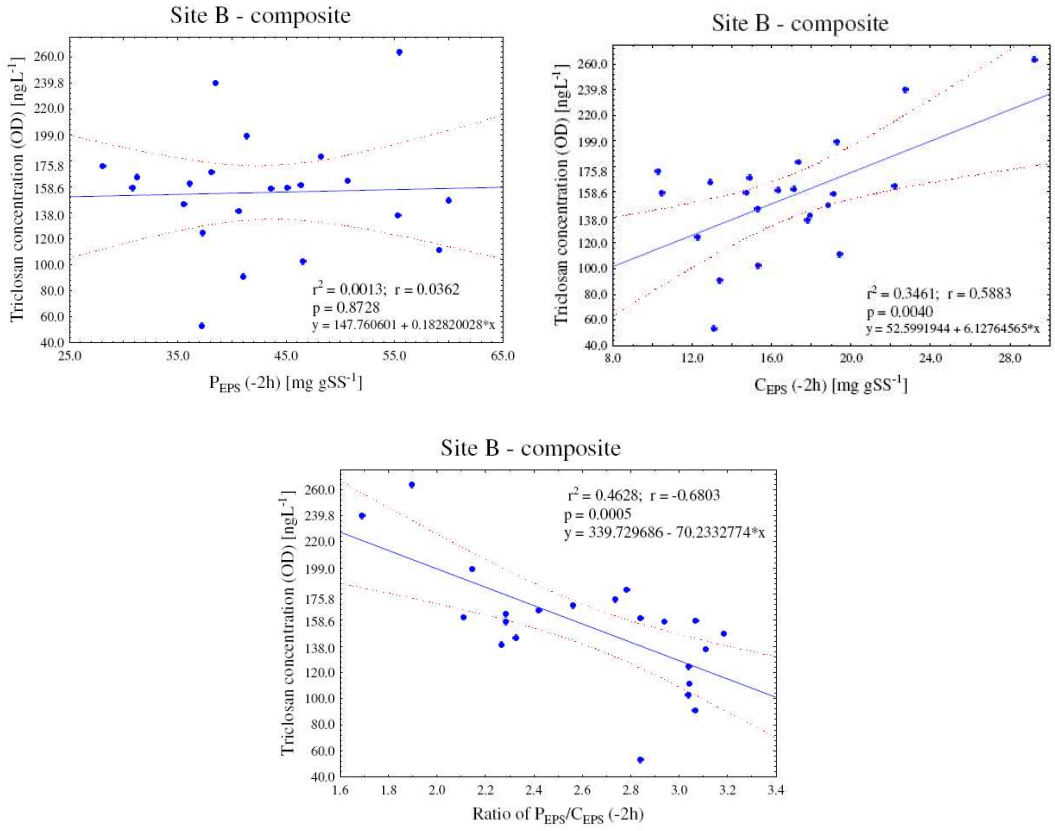


Figure 5.51: Correlation of P_{EPS}, C_{EPS}, P_{EPS}/C_{EPS} after two hours and Triclosan concentration of the oxidation ditch

Correlating the Triclosan primary removal against concentrations of proteins and carbohydrates of the EPS fraction (*see Figure 5.52*) yielded in similar results. The higher the Triclosan removal rate was, the lower was the P_{EPS} concentration, C_{EPS} respectively. Whereby the C_{EPS} concentration showed little higher significance (*r=-0.6171*) than P_{EPS} (*r=-0.3515*), whereas ratio of P_{EPS}/C_{EPS} showed no numerical correlation (*r=0.3025*).

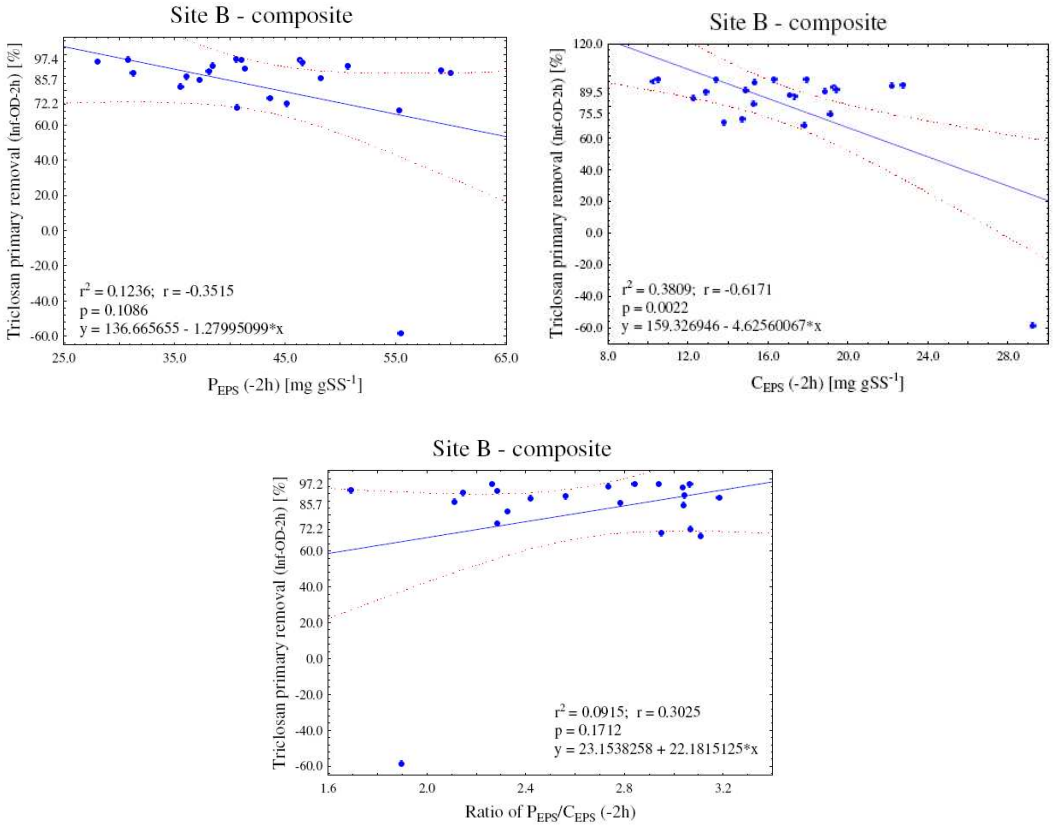


Figure 5.52: Correlation of P_{EPS}, C_{EPS}, P_{EPS}/C_{EPS} after two hours and Primary Removal of Triclosan

Interpreting these results is quite difficult as bioactivity within wastewater treatment is a highly complex process, depending on various uncontrolled factors. Furthermore, no laboratory test results were available while finishing this thesis.

However, biomass was shown to react to increasing concentrations of toxic substances. Schmitt *et al.* (1995) stated higher polysaccharides formation within the EPS at a toluene level of 5 mgL^{-1} and at a level of 15 mgL^{-1} the formation of carboxyl-groups. Therefore, lipophilic compounds might change the make-up of the biomass and in the case of toluene increasing the amount of negatively charged groups, responding in, for instance, more binding sites for metal-compounds. However, the concentrations of Triclosan within influent and effluent samples were far below the mgL^{-1} range, and no studies have been conducted concerning other compounds which might also have an impact on biomass-changes.

Noteworthy is the fact that EPS Protein and Carbohydrate concentrations showed no correlations to the sorbed amount of Triclosan within the EPS, nor did the ratio of P_{EPS}/C_{EPS}. Protein and Carbohydrates concentration within the bulk phase did neither result in any numerical correlation to the Triclosan uptake within the EPS.

5.3.7 Suspended Solids and Volatile Suspended Solids

Triclosan is suspect to sorb relatively quickly to suspended solids due to its high organic-carbon coefficient, K_{oc} of 47,500 Lg^{-1}(Ciba Speciality Chemicals, 2001*b*).

Determination of suspended solids for samples other than the activated sludge, such as influent and effluent have only been conducted for the 48-hour monitoring sampling and therefore no results from the grab sampling period will be represented.

5.3.7.1 48-hour monitoring sampling

Values of suspended solids [mgL^{-1}] and volatile solids [%] for influent (*B1*), oxidation ditch (*B2*) and final effluent (*B3*) are shown in Figure 5.53 and Figure 5.54. The high variation in volatile solids could be attributed to the analytical method, which allowed no measurement replicates due to the low content of suspended solids.

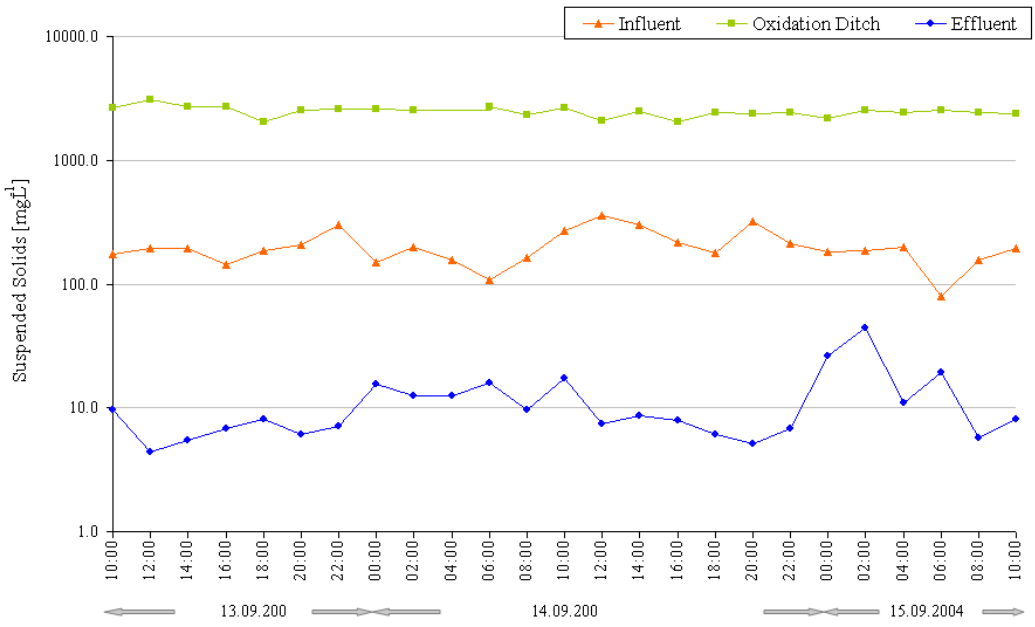


Figure 5.53: Suspended Solids values - 48 hour monitoring - Site B

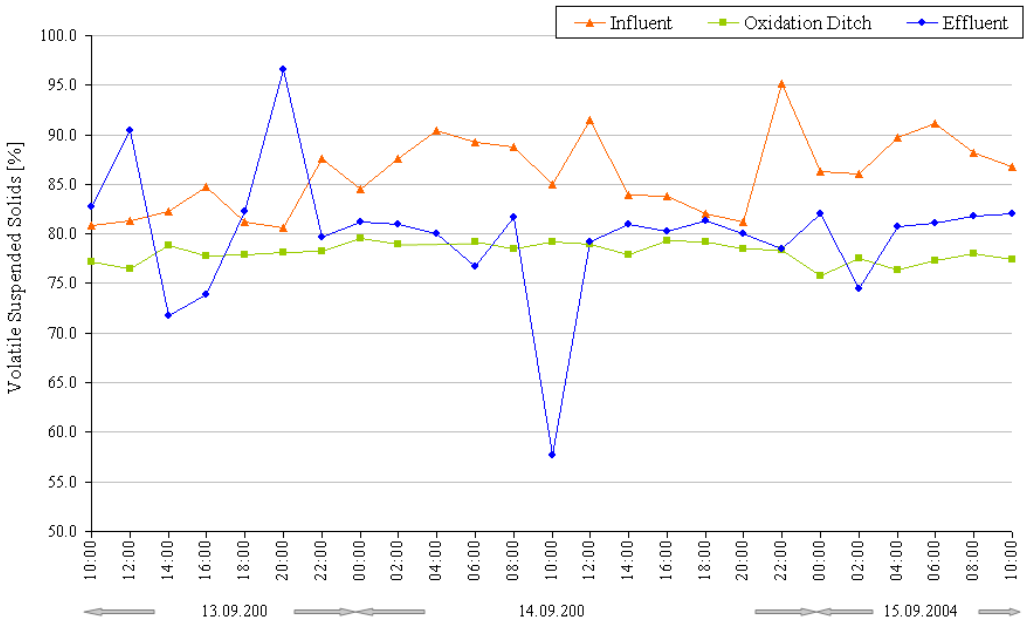
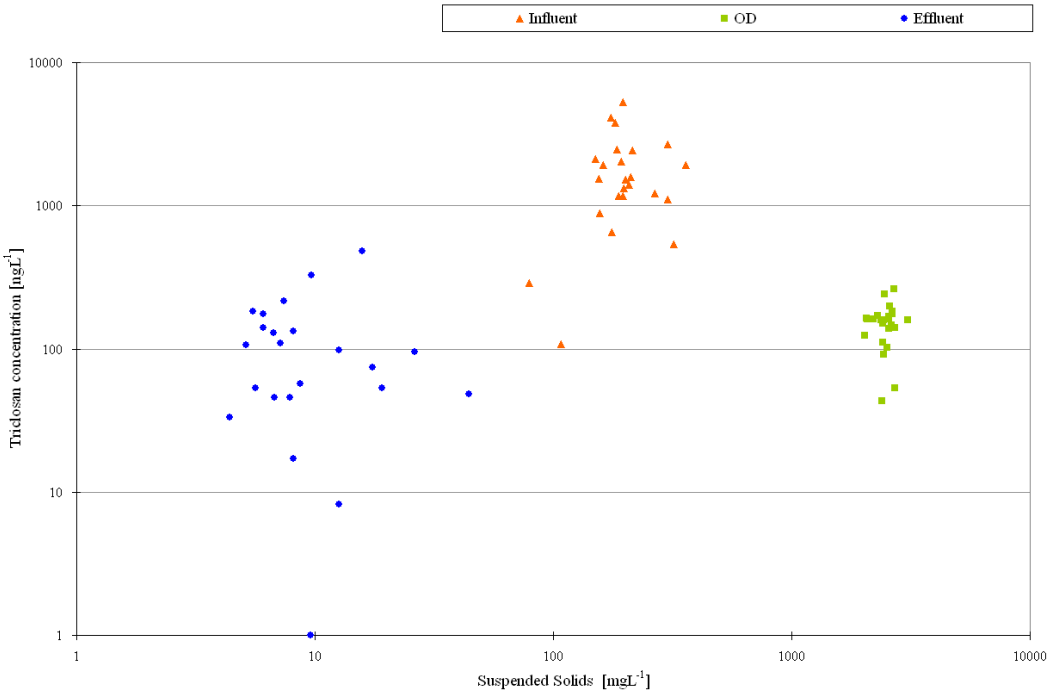
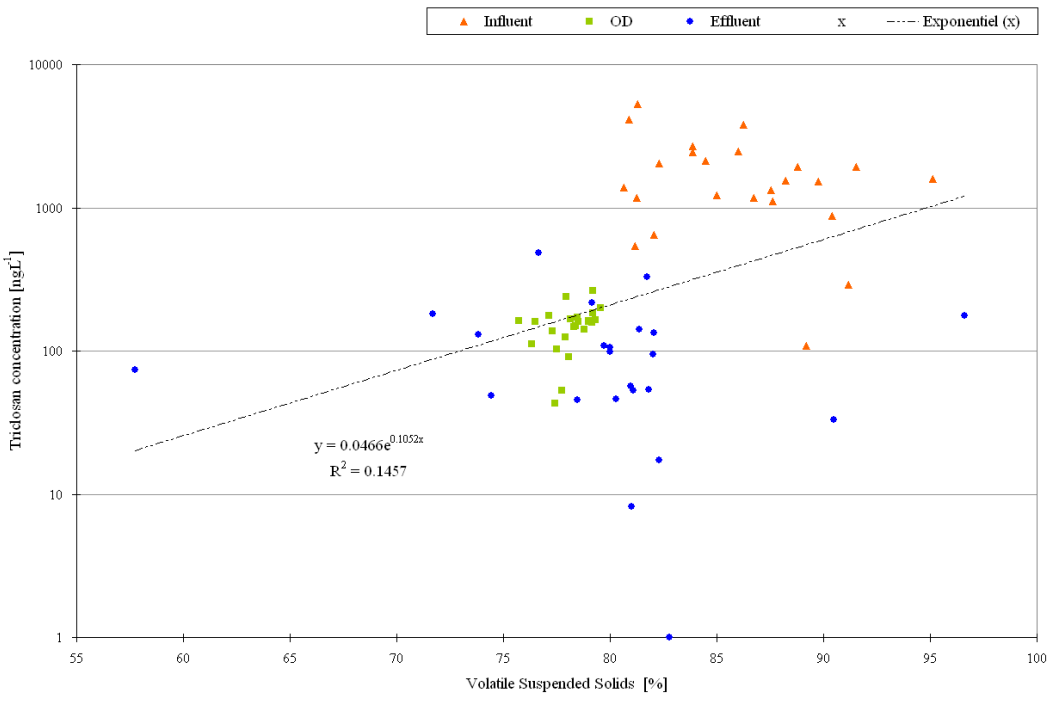


Figure 5.54: Volatile Suspended Solids values - 48 hour monitoring - Site B

No numerical correlation was observed between the amount or removal rate of suspended solids and volatile suspended solids with Triclosan concentration or removal rate. Correlation of concentration data of Triclosan within each treatment stage to data of Suspended Solids, Volatile Suspended Solids respectively, are shown in Figure 5.55.



(a)



(b)

Figure 5.55: Triclosan concentration vs *(a)* Suspended Solids [mgL^{-1}] and *(b)* Volatile Suspended Solids [%] values - 48 hour monitoring - Site B

It should however be noted that Triclosan values have only been measured for solved compounds (filtrated supernatants) and no aliquots have been determined for Triclosan amounts sorbed to particles in the influent or effluent samples. For instance, Schneider *et al.* (2004*a*) stated a high affinity of Triclosan to be bound to the particle phase.

Sorption processes within wastewater can be estimated with the help of the sorption coefficient (K_d), a value depending mainly from the characteristics of the compound as well as of the sludge (Ternes *et al.*, 2004). Studies conducted within the POSEIDON project did not reveal any correlation between the K_d value with literature values, such as octanol water partitioning K_{OW} or partitioning to soil organic carbon K_{OC} (Ternes *et al.*, 2004).

As no laboratory results about the K_d value for Triclosan and the activated sludge of the oxidation ditch have been available for this thesis and therefore no correlations could be done regarding the estimated values of sorbed Triclosan and the determined content of Triclosan within the EPS fraction.

In fact, the found Triclosan contents in the EPS fraction did show rather a negative correlation to suspended solids, volatile suspended solids [mgL^{-1}] within the oxidation ditch. Figure 5.56 shows the STATISTICA graphs for suspended solids (*r=-0.3800*) and volatile suspended solids (*r=-0.3682*) correlation to the Triclosan concentration within the EPS fraction.

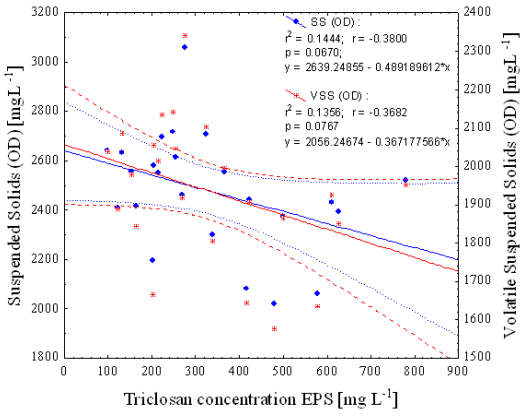


Figure 5.56: Correlation of SS (OD) and VSS(OD) vs Triclosan concentration of the EPS fraction

5.3.8 Particle size and specific surface

Due to the fact that determination of Triclosan within the EPS fraction could not be done for the grab sampling period, results for particle size and hence comparison to Triclosan uptake within the EPS will only be presented for the 48-hour monitoring study.

5.3.8.1 48-hour monitoring sampling

Fair correlation was found between EPS Triclosan content and both the **particle size** (*r=0.4635*) and **specific surface** (*r=-0.4351*). Therefore indicating that Triclosan tends to adsorb better to bigger sludge flocs, even though these observations are not very significant and results might be influenced by other factors. Due to missing lipid determination it cannot be concluded whether any other impact of the make up of the sludge flocs might be alter the sorption of Triclosan.

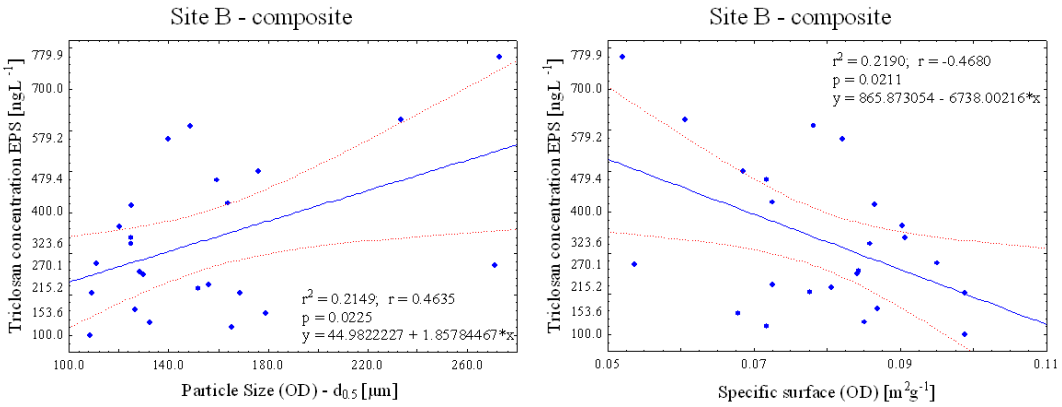


Figure 5.57: Correlation between Triclosan content in EPS fraction and particle size and specific surface

Interestingly, the determined values for particle size, specific surface respectively, of the biomass of the oxidation ditch showed also fair correlation to Triclosan effluent concentrations after two hours (*r=0.5207 for particle size, r=-0.4692 for specific surface*). Statistical graphs are shown in Figure 5.58.

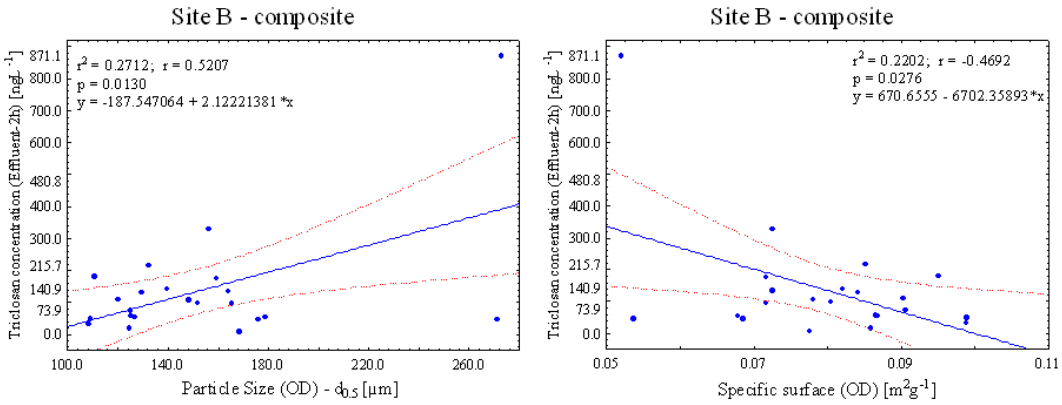


Figure 5.58: Correlation between Triclosan effluent concentration after two hours and particle size and specific surface of the oxidation ditch

This observation might fit into the assumption for the increased Triclosan effluent concentration due to re-balancing effects within the final clarifier. Sludge flocs in the oxidation ditch with higher particle size, lower specific surface respectively, seem to tend to higher Triclosan uptake within the EPS for bigger sludge flocs of the oxidation ditch. These bigger sludge flocs, once in the final clarifier, seemed to release the Triclosan bound to the EPS bound and hence leading to increased Triclosan effluent concentrations. However, it should be noted that correlations were rather fair than significant and sorption and desorption processes might depend also on other (probably not determined) parameters.

5.3.9 Nitrate, Nitrite, Phosphate and Sulfate

Nitrate, nitrite, phosphate and sulfate concentration have been measured using a Dionex Ion Chromatograph and analyses were only undertaken for the 48-hour monitoring composite sampling. Samples were prepared and stored frozen due to a technical failure with the chromatograph.

As the method had been adapted from clean water analysis, the chromatograms showed overlapping peaks for sulfate and phosphate and results are therefore missing. Furthermore, the results for nitrite and nitrate seemed also to be influenced by the problems which occurred within the chromatograms and the gained data revealed no correlation at all to either Triclosan concentration or removal rates, nor did they seem to have any impact on protein and carbohydrate concentration, ratio respectively. Data values can be found in the full scale sampling list of the 48-hour monitoring and will therefore not be presented within this section.

5.3.10 Lipids

Triclosan is suspected to enrich itself better to lipid containing sludges due to its hydrophobic character. Antusch (1999) demonstrated that sorption of phenols onto activated sludge and sewer biofilms increased with increasing lipid content and with decreasing pH. This is expected to be similar for Triclosan due to its hydrophobic and phenolic character.

The standard method for lipid determination by APHA (1998) requires a quite high amount of sludge sample, which has not been available due to sampling stratifications. Lipid determination has been therefore carried out using freeze dried sludge samples in connection with sludge extraction with an adapted method by Merck (1974). Aliquots were taken from the hexane-isopropanol extract and a colourimetry analysis for lipid contents was done (Schettgen, 2000).

Due to time limitation it was not possible to determine any sludge sample of the composite sampling by the Merck (1974) method, where comparison could have been drawn from results of lipid determination conducted by A. Thompson with the standard method by APHA (1998). Furthermore, the lipid results of the 48 hour monitoring sampling conducted by A. Thompson with the standard method by APHA (1998) have not been available for this thesis.

5.3.10.1 Grab samples

Table 5.9 provides the determined lipid content from Site A,B,D for the grab sampling period.

Table 5.9: Lipid content - Site A, B, D

Site	type of sludge sample	lipid content [μg g dm]
A	A5 - fixed biofilm (*RBC*)	327 - 670 (*529*)
B	B2 - suspended solids (*oxidation ditch*)	486 - 783 (*600*)
D	D4 - suspended solids (*anoxic lane*)	256 - 460 (*358*)
	D5 - suspended solids (*aerob lane*)	255 - 421 (*338*)
	D6 - suspended solids (*RAS*)	183 - 337 (*260*)

Interestingly, the oxidation ditch showed higher lipid content than the fixed biofilms which was not expected. However, this might be due to the fact that Site B does not have a primary settler, where usually high lipid containing sludge is removed. Another fact is that, unfortunately it has not been possible to compare the standard method by APHA

(1998) and the tried method by Merck (1974) by analysing aliquots of sludge samples. This should be undertaken before suggesting any reliability and efficiency of the method and hence results for lipid contents.

All samples determined for their lipid content seemed to correlate significantly with the overall Triclosan removal, though correlation for Site D could just be undertaken with two data pairs. The STATISTICA graphs for the grab sampling period are given in Figure 5.59.

The graphs in Figure 5.59 might verify the suggestion that increasing lipid content of the biomass enhanced the Triclosan removal, probably due to sorption processes of the lipophilic compound Triclosan. This might explain also the higher removal rates of Triclosan within the primary settler of Site C and Site D.

However, it should be noted again that samples for the grab sampling period do not correlate with the hydraulic flow and for Site D only two valid pairs of samples existed for the lipid correlations. Furthermore, the used method by Merck (1974) should be tested in laboratory test for its reliability, which has not been possible for this thesis.

As the results of lipid contents for the sludge samples within the 48-hour monitoring period were not available for this thesis, it would be necessary to determine in further reports, if there are any correlations to the Triclosan content of the EPS.

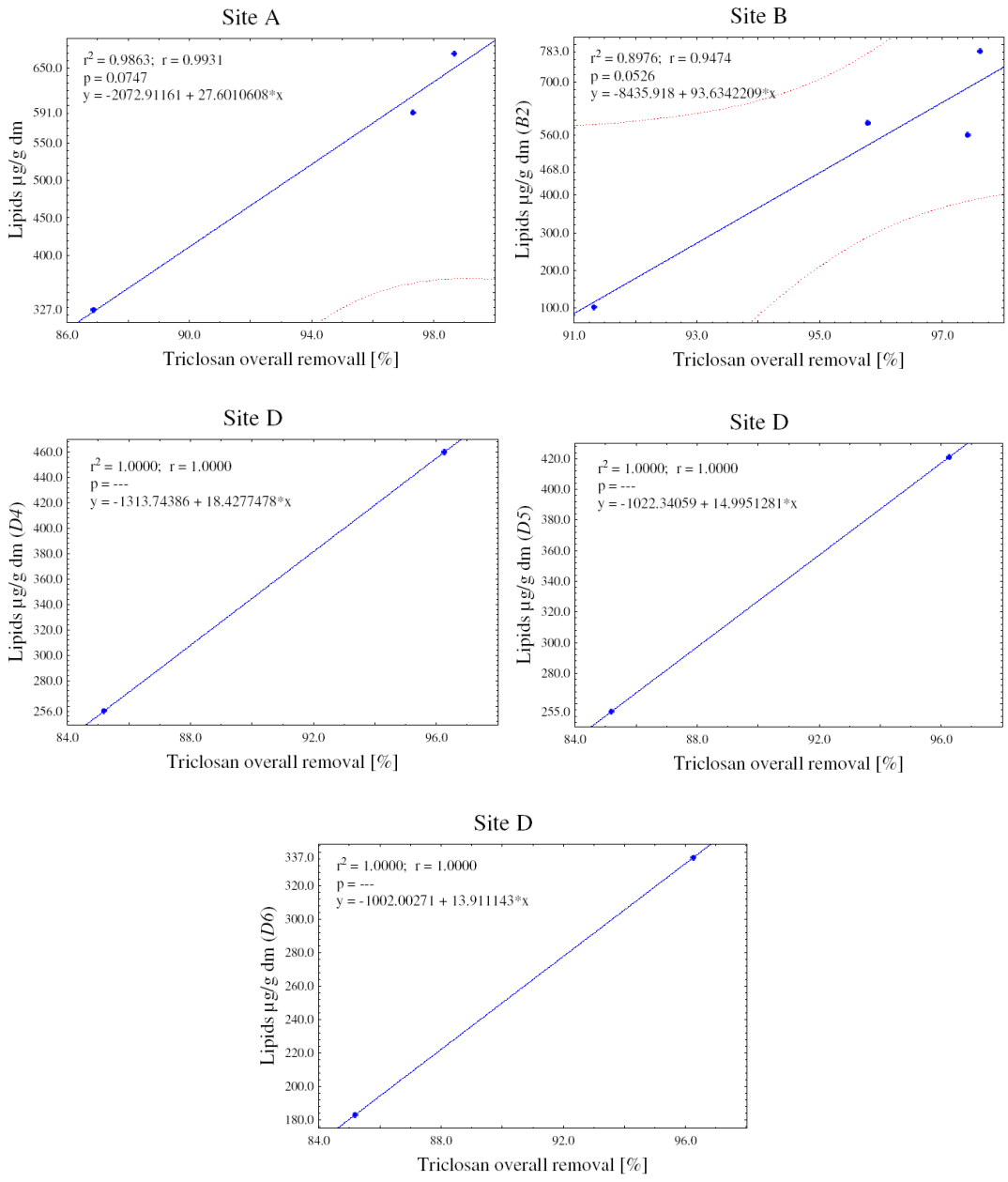


Figure 5.59: Correlation of lipid content of the sampled biomass to Triclosan overall removal - Site A,B,C,D

6 Discussion

6.1 Triclosan

6.1.1 Comparison of Site A, B, C, D

It is quite difficult to compare all sampling sites as they are subject to widely varying conditions, especially as it is not recommended in theory to compare wastewater treatment plants with different influent conditions. It is also difficult to compare the individual dates as most of the samples, except for the monitoring study of site B, represent grab samples without any hydraulic flow correlation.

However, Triclosan was removed within all four sampled sites with an average removal of 81% *(Site A)* up to 97 % *(Site B)*. This was expected as Triclosan was reported to be readily biodegradable or to show high affinity for removal due to sorption onto suspended solids (McAvoy *et al.*, 2002; Singer *et al.*, 2002; Bester, 2003; Kuch *et al.*, 2003). The Oxidation Ditch exhibits the highest and most consistent average removal. This was expected due to the high hydraulic retention time of average of 30h and therefore allowed the biomass to adapt to changing influent conditions (Federle *et al.*, 2002; Dokianakis *et al.*, 2004; Clara *et al.*, 2004).

Table 6.1 provides an overview of the examined wastewater treatment systems and their average overall removal regarding Triclosan. The results only include values from this study and are compared to a similar study conducted by Schneider *et al.* (2004*b*), where influent conditions of the WTPs were reported to be equal.

Table 6.1: Comparison of Triclosan removal rates

Wastewater treatment system	**Site**	**Overall removal within this study**	**Schneider *et al.* (2004*b*)**
Activated Sludge	D	93%	94%
Oxidation Ditch	B	97%	97%
Rotating Biological Contactor	A	81%	93%
Trickling Filter	C	92%	92%

In comparison, the results do not seem to vary much from ideal compared WTPs, except for RBC treatment *(Site A)*. Site A yielded in the highest average concentration of 229 ngL^{-1} for the effluent, but due to the fact that it also has the lowest average flow of just 120 m^3d^{-1}, its final effluent load does not exceed an average of 0.023g day^{-1}, whereas the largest WTP, Site D, has much lower average outflow concentrations of 60 to 138 ngL^{-1}, but due to its immense daily flow rate the average output to the environment yielded in 7.54 g day^{-1}.

An overview of average inflow-outflow load values is given in Table 6.2, while comparison values from selected literature data are given in Table 6.3.

Table 6.2: Triclosan load - Comparison WTP A, B, C, D *(March 04 - Sep 04)*

Wastewater Treatment Plant	**Triclosan load in g day^{-1}**		**Triclosan load in μg day^{-1} per capita**		*Overall Removal Rate* [%]
	Influent	Effluent	Influent	Effluent	
Site A *(RBC)* PE: 405	0.22	0.016	545	68	*(81)*
Site B *(OD)* PE: 13,440	7.4	0.23	551	17	*(97)*
Site C *(TF)* PE: 2,750	2.1	0.16	923	67	*(92)*
Site D *(ASP)* PE: 494,387	147	7.5	296	15	*(93)*

From Table 6.2 and Table 6.3 it can be seen, that the average influent Triclosan load per capita and day of WTP A to D varies between 0.3 to 0.9 mg, and results are similar to studies conducted in the UK (Sabaliunas *et al.*, 2003), Germany (Bester, 2003) and Switzerland (Lindström *et al.*, 2002; Singer *et al.*, 2002), while McAvoy *et al.* (2002) stated an average input rate of Triclosan of 3 to 5 mg per capita and day for WTPs in the United States. These high variations might be suggested to different consumer patterns for Triclosan in Europe and North America.

It should be noted, that the very low daily Triclosan input rates per capita according to the study by Thomas and Foster (2005), are solely based on the fact, that the given numbers for effluent flow rate are extremly low. According to the provided numbers of a total PE of 1,100,000 and a total flow rate of 41,000 m^3day^{-1}, the average effluent flow rate would result in $\sim$ 37 L per capita and day. The resulting extreme low load data are therefore just provided in table 6.3 but not included in the discussion.

Table 6.3: Triclosan load - Comparison of *(estimated*)* values according to selected literature data

Wastewater Treatment Plant	**Triclosan load in g day^{-1}**		**Triclosan load in μg day^{-1} per capita**		*Overall Removal Rate* [%]
	Influent	Effluent	Influent	Effluent	
United Kingdom					
Activated Sludge Plant PE: 8,878 (Sabaliunas *et al.*, 2003)	11.5	0.6	1,293	65	*(95)*
Trickling Filter PE: 7,901 (Sabaliunas *et al.*, 2003)	3.9	0.2	497	23	*(96)*
Germany					
Activated Sludge Plant PE: 350,000 (Bester, 2003)	200 240	8 11	571 686	23 31	*(95)* *(96)*
Switzerland					
Activated Sludge Plant PE: 4,500; 9,200 (Lindström *et al.*, 2002)	3.4 3.3	1.9 0.8	756 359	422 87	*(44)* *(76)*
Activated Sludge Plant PE: 10,500; 10,500; 11,000 (Lindström *et al.*, 2002)	2.0 3.2 3.4	0.3 0.3 0.5	190 305 309	29 29 45	*(85)* *(91)* *(85)*
Activated Sludge Plant PE: 10,500 (Singer *et al.*, 2002)	6.7	0.4	638	38	*(94)*
Activated Sludge Plant PE: 19,000; 36,600 (Lindström *et al.*, 2002)	8.8 18.5	2.7 1.6	463 505	142 44	*(69)* *(91)*
United States					
Activated Sludge Plant PE: 27,000; 398,000 (McAvoy *et al.*, 2002)	128.4 1,109.7	4.9 51.1	4,756 2,788	182 128	*(96)* *(95)*
Trickling Filter PE: 2,445; 3,096; 3096 (McAvoy *et al.*, 2002)	7.2 12.3 16.6	3.1 2.2 2.1	2,976 3,979 5,362	1,251 698 678	*(58)* *(82)* *(87)*
Activated Sludge Plant PE: 194,000; 375,000; 500,000 (Thomas and Foster, 2005)	33.9 45.5 57.2	0.8 0.6 0.4	175 121 114	4 2 1	*(98)* *(99)* *(99)*

* missing values calculated according to given data in literature

In order to compare the pathway Triclosan through the different treatment stages an average mass flow balance of each WTP has been examined of WTP A to D. Appropriated values were selected from the full scale sampling list and Triclosan mass flow was calculated with flow rates of the WTPs. The balance of Triclosan's mass flow in all sites can be seen in Figure 6.1 and is discussed in detail in the following sections.

Site A

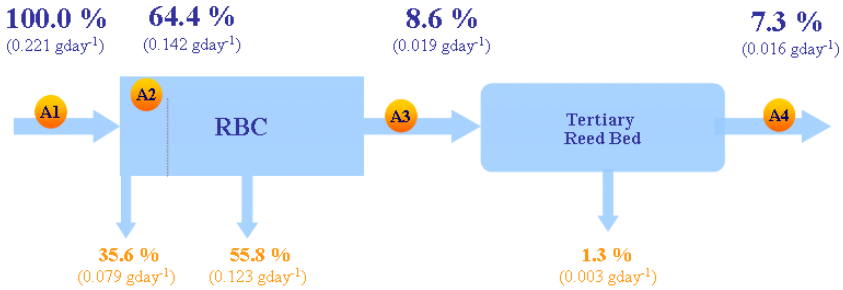
Due to the very low waste water inflow of in average 120 $m^{-3}day^{-1}$ the Triclosan input yielded in only 0.221 g per day at A1 (= 100%) over the monitored period. In normalising to sampling point A1, a significant loss of Triclosan with in average 35% could be observed at sampling point A2. While passing the biological stage, the rotating biological contactor was found to contribute to in average 55.8% of overall Triclosan removal. Surprisingly, no significant loss rate (only 1.3%) was observed for the tertiary reed bed. On average 7.3% of the total incoming Triclosan mass flow left this WTP at A4, corresponding to 0.016 g Triclosan per day.

Site B

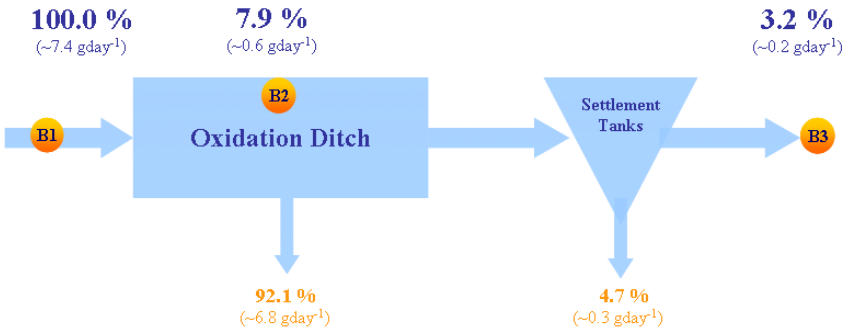
The average Triclosan mass inflow of the oxidation ditch yielded in 7.4 g day^{-1} (B1), over the monitored period This WTP showed the most significant overall removal of all three sites. Only 3.2% of total Triclosan mass inflow left this WTP at the point B3, constituting an average output of 0.2 g Triclosan per day. The major elimination step occurred at the biological stage, the oxidation ditch, with an average triclosan loss rate of 92.1%. This was expected because of the high hydraulic retention time of average 30 hours and a higher sludge age and therefore allowed biomass adaptation to changing influent conditions (Ternes *et al.*, 2003; Federle *et al.*, 2002; Dokianakis *et al.*, 2004; Clara *et al.*, 2004). Compared to the overall removal, the Triclosan loss within the final settlement tank seemed to be relatively small with approx. 4.7% of Triclosan mass loss rate in average.

Taking the higher effluent values from the 48-hour monitoring study into consideration *(see Figures 5.9 and 5.10)*, one could conclude that Triclosan bound to solids is undergoing desorption within the final clarifier due to re-balancing effects. This re-balaning effect was also reported by Schneider *et al.* (2004*a*). Furthermore, Kuch *et al.* (2003) also determined ten different wastewater treatment plants, where three WTPs showed higher effluent values for Triclosan than the influent. This was suggested to be due to stoppages within these sites and also due to re-balancing effects.

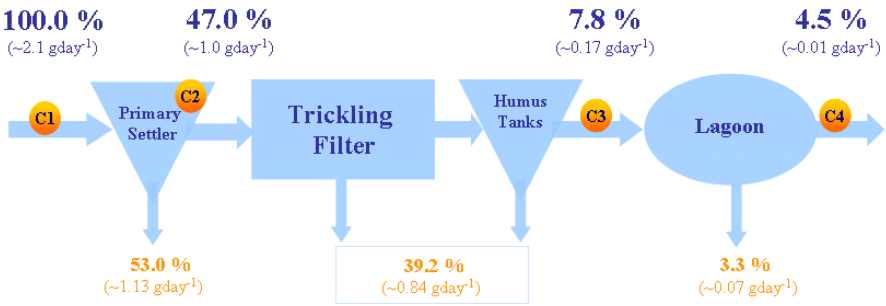
Site A - Rotating Biological Contactor



Site B - Oxidation Ditch



Site C - Trickling Filter



Site D - Activated Sludge Plant (anox/aerob)

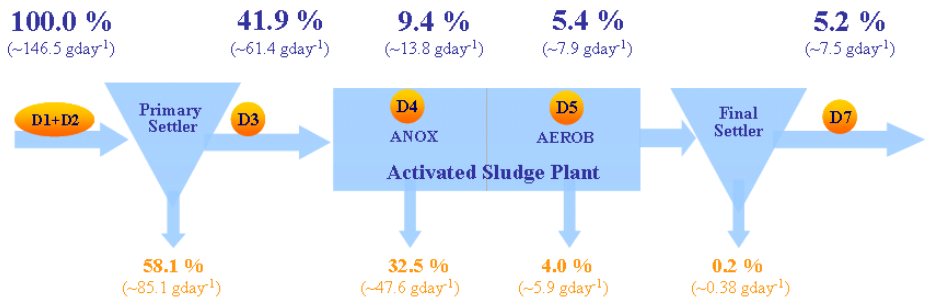


Figure 6.1: Mass flow balances for Triclosan

Site C

The trickling biological filter also demonstrated a very high loss rate of total Triclosan inflow at the primary settling point, where in average over 53% of Triclosan were eliminated from the liquid phase. A probable explanation is that Triclosan is a hydrophobic compound which adsorbs well to the primary sludge, which is known to have relatively high fat content and therefore more hydrophobic binding sites. This occurrence has been, in fact, observed as well within the primary settler of the activated sludge plant (Site D), where almost 58% of triclosan loss rate could be observed within the primary settler. This phenomenon is in accordance with a study conducted by Kuch *et al.* (2003), where significant removal was observed within the primary settlers of sewage treatment plants (up to 60%).

Another major loss rate of approx. 39% occurred after the passage of the trickling filter itself, whereas it cannot be clarified how much within the humus effluent tank is due to elimination or desorption processes respectively, due to sampling techniques.

Approximately 3.3% of the overall Triclosan loss was observed within the polishing lagoon. In fact, this reassembles the observation made for the tertiary reed bed of the RBC, which also contributed just slightly to the overall removal of Triclosan. In total 4.5% of the overall Triclosan input left this WTP, being valued with in average 0.01 g Triclosan per day.

Site D

The activated sludge plant, the biggest determined WTP, showed the highest Triclosan input of all four case study sites, which is obviously due to the much higher PE. In average 146.5 g Triclosan entered this WTP per day. Similar to site C, the primary settler showed a high elimination of Triclosan of 58%. This elimination rate is up to 5% higher than for the primary settler of Site C, which might be due to either higher hydraulic retention time within the settling process, differing influent conditions or a different make-up of the primary sludge (e.g. higher sludge content). Thomas and Foster (2005), for instance, reported a higher elimination rate of Triclosan for an U.S. WTP within the primary clarifier due to the addition of polymer flocculant to the raw influent.

The anoxic treatment stage revealed an average elimination rate of approximately 33%, whereas within the aerobic lane only 4% further elimination of Triclosan occurred. This might lead to the conclusion that denitrifying treatment stages seem to be very efficient with regards to Triclosan removal. In fact, this should be investigated further, perhaps within a WTP of similar size, where the denitrifying treatment stage follows the aerobic lane. Nevertheless, regarding only the biological stages, the activated sludge plant seemed to have one of the lower removal efficiencies of Triclosan from the liquid phase. However,

this might be again have resulted from the sampling stratification and the fact that the influent conditions of the determined WTPs varied widely.

The elimination rate of Triclosan within the final clarifier was shown to be very low with only 0.2%. This was much lower than that observed for the settlement tanks of WTP B, which might be due to sampling techniques or sampling stratification. Again, re-balancing effects of Triclosan within the final settler might also have an impact on the low elimination efficiency. Almost 5.2% of all inflowing Triclosan were found to leave this WTP in the liquid phase, representing a daily output of approximately 7.5 g Triclosan.

Daily Triclosan output values

The daily output of all determined WTPs varied for the two fixed bed film sites between 22 to 95 μg Triclosan per capita and day (RBC) and from 15 to 126 μ per capita and day (TF). Due to the high overall removal rate the daily Triclosan output for the suspended growth system, the oxidation ditch was just between 1 to 41 μg per capita and day. The activated sludge plant showed a daily output from 15 to 94 μg Triclosan per capita.

These values are comparable to other studies (see Table 6.3). Singer *et al.* (2002), for instance, stated for Swiss WTPs a variation of 30 to 210 μg per capita and day and Paxéus (2004) reported in a comparison study between 10 WTPs in Europe an average output per capita and day of 0.15 mg ($\pm$ 0.08 mg) Triclosan. The daily output for the WTPs in the United States examined by McAvoy *et al.* (2002) resulted in an average daily output of 0.15 mg Triclosan for the activated sludge plants, whereas the trickling filter plant were reported to have an average daily output of 0.88 mg Triclosan per capita. The highest reported daily output of Triclosan per capita was 1.2 mg (McAvoy *et al.*, 2002).

An overview of annual estimated values per capita for Triclosan usage, emission respectively, for different countries are given in Table 6.4.

Table 6.4: Estimated annual Triclosan values per capita - usage and emission

Country	**Annual values of Triclosan in g per capita and year**		**Referenz**
	Annual Usage	Average Emission	
United Kingdom	0.231	0.017	*this study*
	0.327	0.016	Sabaliunas *et al.* (2003)
Germany	0.229	0.010	Bester (2003)
	0.895	-	Bester (2005)
Switzerland	0.233	0.014	Singer *et al.* (2002)
	0.144	0.042	Lindström *et al.* (2002)
United States	1.38	0.06	McAvoy *et al.* (2002)
	1.50	0.32	

6.1.2 Comparison to other studies

At the present time, only few records exist with regards to the monitoring Triclosan through wastewater treatment plants at different treatment stages. Figure 6.2 shows data gained from literature from WTPs in the United Kingdom (Sabaliunas *et al.*, 2003), the United States (Thomas and Foster, 2005; McAvoy *et al.*, 2002) and Germany (Kuch *et al.*, 2003) in comparison to the results found within this study. It should be noted that data from literature were calculated from given Triclosan concentrations, whereas the numbers from this study were taken from the mass flow balance. Therefore, the overall removal rates are not conclusively comparable.

However, Figure 6.2 indicates well the difficulties in comparison different WTPs with widely varying conditions. So, for instance, influent concentrations are likely to be variable because they are dependent upon various factors such as location, socioeconomic status, pharmaceutical cost, consumer patterns and other demographic data (Thomas and Foster, 2005). Data for micropollutants may vary widely even for the same sample due to different analytical methods. Sampling stratification is another important factors for reliable comparison of different wastewater analyses.

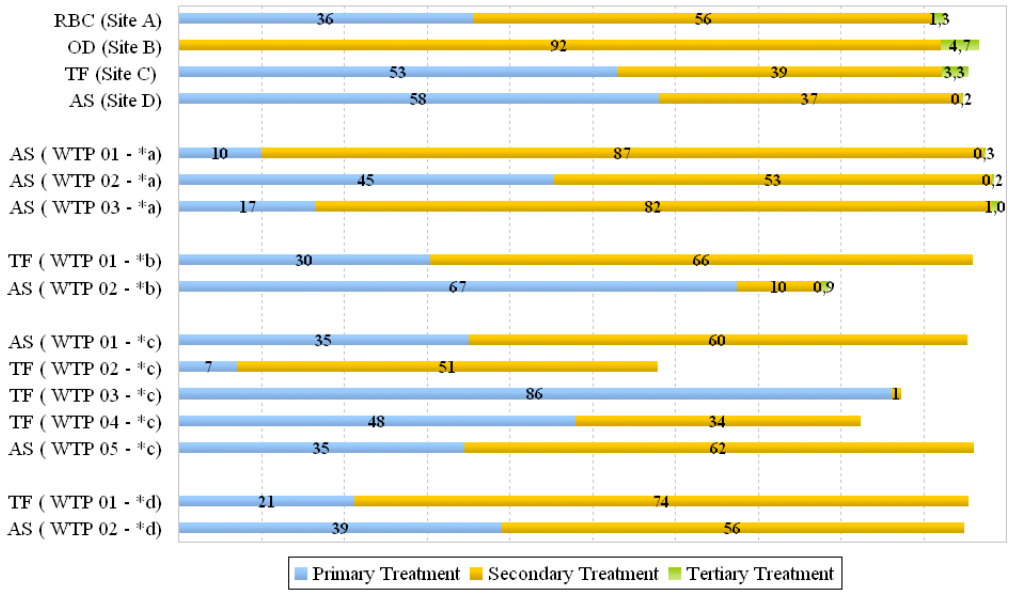


Figure 6.2: Elimination rates for Triclosan within treatment stages of different WTPs
AS - Activated Sludge, TF - Trickling Filter, OD - Oxidation Ditch, RBC - Rotating Biological Contactor
*a Thomas and Foster (2005), *b Kuch *et al.* (2003), *c McAvoy *et al.* (2002), *d Sabaliunas *et al.* (2003)

Within this study the sorption of Triclosan to suspended solids, especially to the higher fat containing primary sludge, and therefore settling processes within the **primary settler** seemed to have a significant influence (up to 53% - *Site C* and ∼58% Site D). This was also reported by Kuch *et al.* (2003) and calculations in Figure 6.2 show a elimination rate of almost 67% for the primary settler of WTP 02 (*b) (Kuch *et al.*, 2003).

One WTP determined by McAvoy *et al.* (2002) even indicated an outstanding elimination rate of approximately 86% of Triclosan within the primary settling process. This is a very high result and might be suggested to the very high influent concentrations of Triclosan of almost 16 μgL^{-1}, but no specific details are given about the primary settler or other important influent conditions, such as, e.g., wastewater parameters or fat content of the primary sludge.

Other primary settlers did not show such high elimination rates compared to the overall elimination rates. Data vary between 7% to 21% and 30% to 48% Triclosan elimination. Thomas and Foster (2005), for instance, observed a minor impact of primary settlers. Rates were for two WTPs *(WTP 01 - *a, WTP 03 - *a)* only 7% and 10%. Interestingly another determined WTP *(WTP 02 - *a)* showed an elimination efficiency of 45% for Triclosan within the primary settler, where a polymer flocculant was added to the raw influent, enhancing primary flocculation processes and probably increasing Triclosan elimination meanwhile.

Nevertheless, **biological treatment** showed in almost all monitored WTPS another major impact regarding Triclosan removal. This was expected, as Triclosan is known to be readily available for biodegradation. However, variations between each site are again very high.

Removal rates from the liquid phase were within this study from ∼ 37% for the activated sludge plant *(Site D)* to ∼ 92% for the oxidation ditch *(Site B)*. For the fixed film treatment processes, removal rates were found to be in average ∼ 39% for the trickling filter *(Site C)* and ∼ 56% for the RBC *(Site A)*.

Thomas and Foster (2005) concluded that the majority of compound removal of 53 to 87% occurred during secondary treatment, while all examined WTPs represented activated sludge processes. It is also worth noting that for other determined pharmaceuticals, such as ibuprofen, caffeine, naproxen, ketoprofen and diclofenac, the secondary treatment was reported to play the major role for compound removal (51 to 99%).

Elimination rates for Triclosan within fixed film treatment stages vary widely from only 1% *(WTP 03 - *c)* to 74% *(WTP 01 - *d)*. Within the study conducted by Kuch *et al.* (2003) the TF plant was reported to show the highest overall removal amongst the examined WTPs.

Final clarifiers have not been determined in most studies. However, results for the final clarifying processes often show little influence on Triclosan overall removal rate *(Site D, 0.2%, Site B, 4.7%)*. Similar observations were made within this study for tertiary treatment, such as red bed *(Site A, appr. 1,3%)* and polishing lagoon *(Site C, appr. 3.3%)*.

In fact, it has been shown during the monitoring study, that final clarifier might have a negative impact on Triclosans overall removal, increasing effluent concentrations of Triclosan. This can be concluded from Figure 5.9 and Figure 5.10, where effluent values, higher than within the liquid phase of the oxidation ditch, were found to be possibly influenced by Triclosan bound to EPS. Correlating the determined Triclosan concentration within the EPS to the Triclosan concentration within the effluent after 2 hours *Eff (-2h)* resulted in significance of r=0.5387 (*p=0.0097*, see Figure 6.3 *(a)*). Correlations for Triclosan content within the EPS (ng Triclosan in EPS per gSS and per gVSS, respectively) were found to be slightly lower (*r=0.4593, p=0.0351 (per gSS) and r=0.4677, p=0.0282 (per gVSS)* - see Figure 6.3 *(b_1) and (b_2)*). Even though this might be an explanation for increased Triclosan effluent values, it should be noted that the found correlations showed to be impacted by one very high effluent value for Triclosan ($C_{Triclosan,Effluent}$=*871 ngL*$^{-1}$).

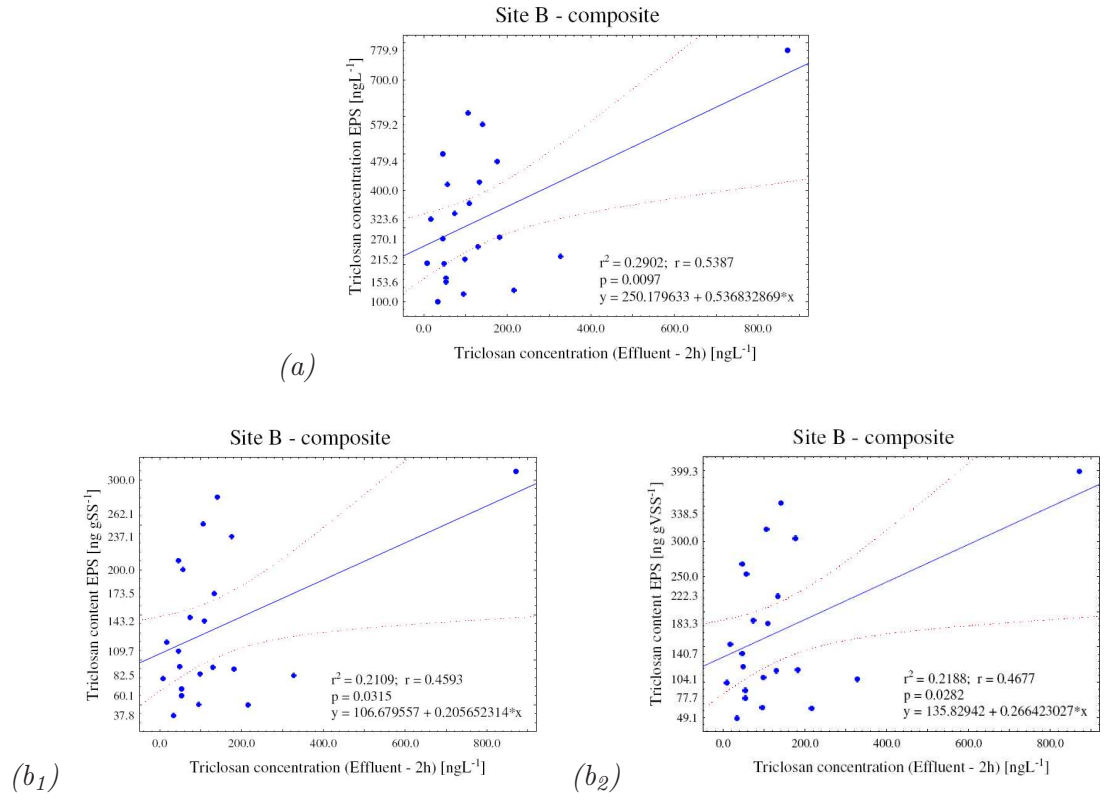


Figure 6.3: Impact of EPS Triclosan concentration/content on effluent concentration after 2 hours *(Effluent-2h)*
(a) Triclosan EPS [ngL^{-1}] vs. Triclosan Effluent after 2h [ngL^{-1}]
(b_1) Triclosan EPS [ng gSS^{-1}] vs. Triclosan Effluent after 2h [ngL^{-1}]
(b_2) Triclosan EPS [ng gVSS^{-1}] vs. Triclosan Effluent after 2h [ngL^{-1}]

Within the grab sampling negative results found for Site A, C and D were linked to sampling stratification and therefore those negative values were left out for the mass flow balance. According to Kuch *et al.* (2003) three out of ten determined WTP were found to have higher effluent values for Triclosan than influent values to the final clarifier *(sites not given in Figure 6.2)*. One suggestion for this phenomenon was the failures at two sites, whereas higher effluent values for Triclosan were also linked to possible **re-balancing** effects (Kuch *et al.*, 2003).

According to Schneider *et al.* (2004*a*), Triclosan can be found bound to solid forms which are easily released within the final clarifier, which leads to no further reduction of Triclosan within the final clarifier and might even lead to higher effluent values (Kuch *et al.*, 2003; Schneider *et al.*, 2004*a*). This was also observed by Bester (2005), where the final clarifying step seemed to result in a "negative" removal of almost 7.5 % of the overall Triclosan input.

Taking hence the negative results found for Site D into consideration and assuming that those negative results would have been caused by re-balancing effects within the final clarifier as been observed for Site B, the mass flow balance would yield in a negative impact of -0.9 % for the final clarifier of Site D (see Figure 6.4).

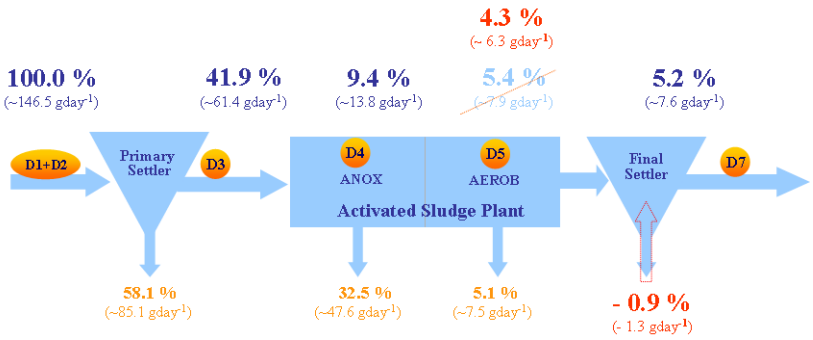
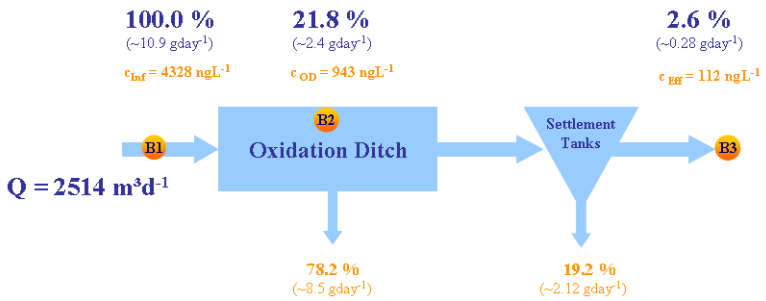


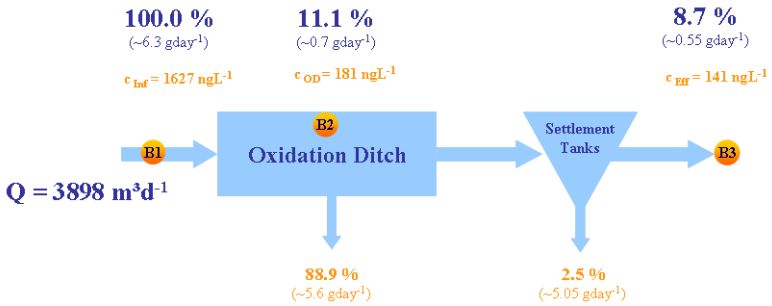
Figure 6.4: Mass Flow diagram including possible re-balancing values for Triclosan within the final clarifier - Site D

6.1.3 Impact of flow rate

McAvoy *et al.* (2002) observed for the trickling filter plant *(WTP 02 - *c)* a very poor removal which was thought to be due to higher water usage rate resulting in a lower hydraulic retention time in the primary clarifier. In fact, within the 48-hour monitoring study for Site B, higher flow rates were suggested to have a negative impact on Triclosans overall removal, which might have occurred due to reduced hydraulic retention time within the final clarifier. The data for sampling of the 09th of September *(Sep 04 (1))* showed much higher influent load, but just half of the effluent load than the value for *(Sep 04 (2)) (see table 5.5)*. In order for better comparison, mass flow data with flow rates for the two occasions are given in Figure 6.5.



(a)



(b)

Figure 6.5: Impact of flow rate on Triclosan overall removal rate - *(a) (Sep 04 (1))* with flow rate at 2514 $m^{-3}day^{-1}$, *(b) (Sep 04 (2))* with flow rate at 3898 $m^{-3}day^{-1}$

As it can be seen in Figure 6.5, the removal rates of Triclosan in the oxidation ditch for both occasions are slightly lower than within the overall observed removal from the grab sampling period *(∼ 92% elimination rate within the oxidation ditch, see Figure 6.1)*. This might resulted from the sampling stratification, where samples usually could not be taken according to the hydraulic flow.

However, Triclosan overall removal rate for the lower flow rate *(Sep 04 (1),∼ 97.4%)* was found to be much higher than the overall removal rate for the higher flow rate *(Sep 04 (2), ∼ 91.3%)*. This seemed mainly to result from the decreased Triclosan removal within the final clarifier, and therefore increasing Triclosan effluent concentration. Almost 19.2% of Triclosan mass input were retained within the final clarifier at the lower flow rate *(Sep 04 (1), Figure 6.5 (a))* resulting in a final effluent output of only 2.6% of Triclosan. In contrary only approx. 2.5% of Triclosan mass input was eliminated within the final clarifier at higher hydraulic load *(Sep 04 (2), Figure 6.5 (a))* leading to increased effluent output of Triclosan of 8.7%.

However, it has to be noted, that the influent concentrations showed significant variations for both sampling occasions *(Sep 04 (1) - 4328 ngL^{-1} and Sep 04 (2) - 1627 ngL^{-1})*, while

the effluent values did not vary that significantly (*Sep 04 (1) - 112 ngL^{-1} and Sep 04 (2) - 141 ngL^{-1}).* Though the measured effluent concentrations within this study ranged from 4 to 141 ngL^{-1}, and showed no consistent value, it should be noted that Kuch *et al.* (2003) observed a 'threshold' concentration value of $\sim$ 40 ngL^{-1} for the removal of Triclosan within wastewater treatment.

Interestingly, the oxidation ditch showed for the sampling on *Sep 04 (1)* the highest ever observed concentration value for Triclosan within the bulk phase *(c = 943 ngL^{-1}).* Therefore leading to the low removal rate within the oxidation ditch for that sampling occasion *(average bulk phase concentration value for the grab sampling period was $\sim$ 100 ngL^{-1})*, whereas the final effluent concentration was found to be lower than for the composite sampling *Sep 04 (2).*

6.2 Wastewater and biomass characteristics

Biological wastewater treatment is a highly complex process depending on various influences, such as temperature, pH, available substrates & enzymes, available oxygen (dissolved or as nitrate oxygen), competitive acting microorganisms, inhibiting or toxic substances. Furthermore the design of the wastewater treatment plant plays a crucial role, influencing the biological wastewater treatment due to various factors.

Within this study wastewater and biomass characteristics were determined from full scale wastewater treatment plants with different biological treatment stages and results were compared to the found Triclosan concentrations, removal rates respectively. Due to the missing sludge extraction and the missing determination of degradation products it is difficult to conclude from this study if Triclosan is either be sorbed to solids or degraded.

Correlations of wastewater and biomass characteristics to Triclosan concentration, removal respectively, were mostly found to be weak to moderate. This might be due to various uncontrolled parameters occurring within full scale wastewater treatment plants. Furthermore, wastewater conditions are known to vary hourly, which leads to the fact, that the correlations of the grab sampling periods ought to be handled with care. Therefore, mostly results from the 48-hour monitoring period will be discussed within this chapter.

Temperature, pH and lipid content

However, lower **pH** values within the primary settler, rising **temperature** and increasing **lipid content** seemed to have significant impact on increasing Triclosan removal within the grab sampling period. This is most likely due to Triclosans chemico-physical properties (*Triclosan tends to be more hydrophobic, if pH « pK_a and high lipid content may enhance hydrophobic interactions*) and the fact, that Triclosan is known to readily biodegraded and therefore rising temperature might enhance biodegradation.

The relevant impact of **pH changes** on the Triclosan removal might be in accordance with other studies, where changing pH affected the water-solid-partition coefficient (Kuch *et al.*, 2003). Therefore, the higher the pH value is above the pK_a of the compound, the more likely it is to be found soluble in the liquid phase. However, the impact of boundary conditions on the removal of organic substances has not been scrutinised extensively, yet. The influence of pH values and the prevalence of cations on the sorption process of pentachlorophenol could be verified by Jacobsen *et al.* (1996). The pH plays only a role when the examined substance is subject to ionisation (Baughman and Paris, 1981). Whenever the molecule is in neutral form, hydrophobic reactions are more important for sorption processes. Nevertheless, the pH values within the 48-hour monitoring period showed no significant correlations to Triclosan removal from the liquid phase, which was suggested

to the sampling stratification, where pH measurements could just be undertaken 24 hours after the samples were taken.

According to Siegrist *et al.* (2003) hydrophobic compounds tend to sorb more readily to primary sludge, due to the high lipid content. However, higher removal rates of Triclosan within the primary settler of Site C and D were suggested to the high **lipid contents** of the primary sludge. Unfortunately, no sludge samples of primary settlers have been available. However the lipid determination of secondary sludge and biomass from the fixed film bed reactor showed significant correlations to the Triclosan overall removal within these sites, being higher with increasing sludge content. This is in accordance to other statements. So, Antusch (1999) reported higher Triclosan sorption to sewer films with increasing lipid content. Furthermore, chlorinated organic compounds were found in sewage sludge in concentrations between 15 and 150 mg kg^{-1} (Friege *et al.*, 1989) and Laschka and Trumpp (1991) reported 500 mg kg^{-1} AOX sorption in sewer films, which might have been probably due to high lipid contents of the biomass. Unfortunately, the sludge extraction did not reveal any reliable results and therefore no correlations can be drawn between the lipid content and the Triclosan sorbed to the sludge, biomass respectively.

Results of the lipid determination for the 48-hour monitoring undertaken by A. Thompson have not been available for this thesis. According to Flemming (1995) the most probably accumulation site of lipophilic substances are the lipid membranes and therefore it might be interesting to examine whether there is any correlation between the lipid content of the suspended solids of the oxidation ditch and the sorbed amount of Triclosan to the EPS fraction.

COD, TOC (*COC*) and specific oxygen demand*

Federle *et al.* (2002) stated a first order correlation between Triclosan overall removal and **COD** overall removal for CAS-experiments. Within this study it was of interest, if high COD removal in full scale WTPs may show similar correlations. In fact, overall removal rates for both parameters, Triclosan and COD, did not reveal any significant correlation, except for the non-hydraulic flow correlated samples of Site C.

Within the 48-hour monitoring study this, in fact, might be resulted by varying conditions which occur at full scale plants rather than in laboratory tests. Furthermore, the observed re-balancing effects within the final clarifier lower Triclosan overall removal rates, whereas this does not occur for COD values. However, measured COD values being a summarising parameter for theoretical available nutrients seemed to have impact on the Triclosan concentration within the oxidation ditch and on the Triclosan uptake within the EPS fraction.

From the measured data and an assumption of a two hours reaction of biomass to changing wastewater parameters it might have been concluded that the higher the COD concentration

was within the bulk phase, the higher was the remaining Triclosan concentration of the bulk phase after two hours. Whereas the lower the COD concentration got within the bulk phase, the higher seemed the Triclosan uptake within the EPS after two hours.

This has been observed also for **TOC** values (*colloidal organic carbon*) of influent samples and the ratio of COD to TOC, which has been assumed within this study as the **specific oxygen demand***. Therefore the main conclusion within the 48-hour monitoring can be made from the observation, that Triclosan removal did seem to depend on on influent nutrient concentration rather than on influent Triclosan concentrations.

For a better overview this will be summarised again and discussed with the help of the next two Figures (*see Figure 6.6 and Figure 6.7*).

Figure 6.6 shows the correlation of Triclosan influent concentration to the Triclosan concentration of the bulk phase of the oxidation ditch and Triclosan concentration of the bulk phase (OD-2h) after two hours. It can clearly be seen that there is no numerical correlation between the influent values of Triclosan and the remaining Triclosan concentrations within the bulk phase.

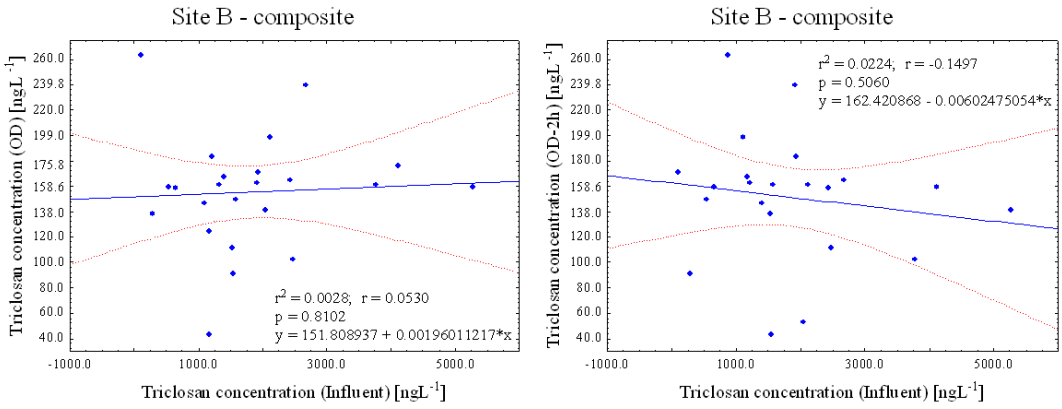


Figure 6.6: Correlation of Influent Triclosan concentrations vs Triclosan concentrations of the bulk phase of the oxidation ditch

The graphs in Figure 6.7 indicate the found correlations between (a) the influent values of TOC, COD and specific oxygen demand* (ratio COD/TOC) to Triclosan concentrations of the oxidation ditch after two hours (OD-2h) and (b) the correlations of wastewater parameters of the bulk phase of the oxidation ditch for TOC, COD, and specific oxygen demand* (COD/TOC) to the Triclosan contents within the EPS fraction after two hours.

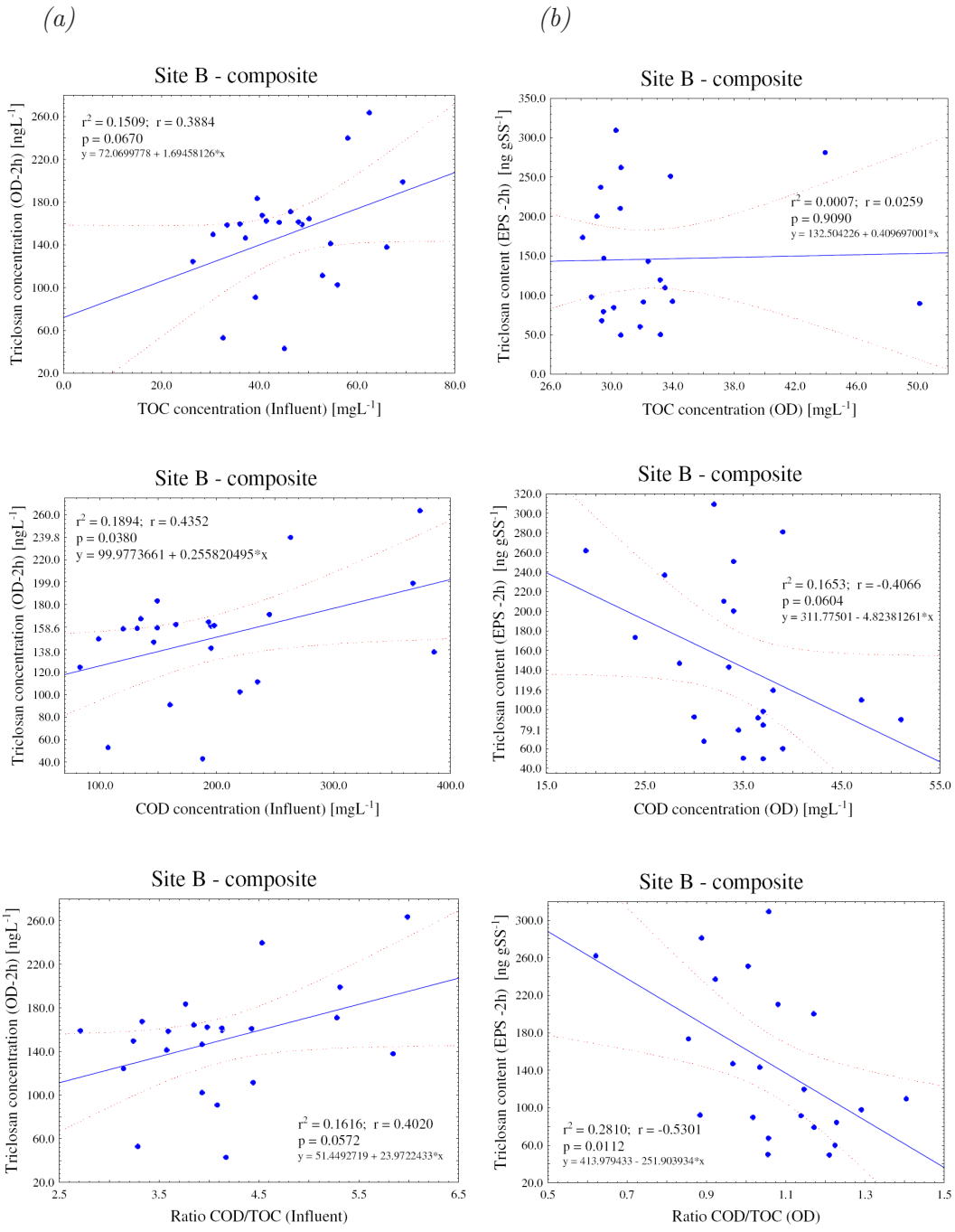


Figure 6.7: Correlation of TOC, COD and ratio COD/TOC of *(a)* Influent to Triclosan concentrations within the bulk phase of oxidation ditch after two hours (OD-2h), and *(b)* oxidation ditch to Triclosan content within the EPS fraction after two hours (EPS-2h) - 48-hour monitoring sampling

From Figure 6.7 it can be seen that Triclosan concentrations remaining in the bulk phase of the oxidation ditch after two hours seem to be higher, the higher the influent values are for COD, TOC and the specific oxygen demand*. Even though the found correlation are more less moderate it might be concluded that Triclosan is taken as secondary substrate whenever there is a shortage of primary substrate.

Furthermore, the graphs for the correlations of COD and specific oxygen demand of the bulk phase to the Triclosan content of the EPS may indicate that the Triclosan uptake within the EPS fraction is enhanced after two hours in case of lower COD concentration and specific oxygen demand* within the bulk phase of the oxidation ditch. Interesting is the fact that the uptake of Triclosan within the EPS did not seem to depend on the TOC concentration of the bulk phase. Therefore, higher EPS uptake seemed to correlate with higher inorganic fractions within the bulk phase.

The oxidation ditch is known to have an intermittent aeration scheme, but as results for nitrite, nitrate, sulfate, phosphorous and chloride were not reliable due to chromatogram failure and as there was no dissolved oxygen measurements available for this study, it can not be concluded on the base of this study, which kind of process might have had an influence on the observed correlations. Nevertheless, it is worth to repeat that the anoxic lane of the activated sludge plant (Site D) showed a higher impact on the loss rate of Triclosan in comparison to the aerob lane, which might have been suggested to the need of additional carbon sources for anoxic treatment processes.

In fact, all observed correlations should have been analysed within laboratory tests, where it is possible to determine boundary conditions and to control influent values. As the laboratory test results were not available while finishing this thesis, no further conclusion can be drawn for either biodegradation or sorption processes of Triclosan within wastewater treatment process.

However, it may be assumed that Triclosan removal within the biological treatment stages seemed mostly to occur due to biodegradation than due to sorption. This may be in accordance to the monitoring study of a full scale plant conducted by Bester (2003), where about 65% of Triclosan seemed to disappear within the treatment process, meaning 65% of incomming Triclosan were not found either in the liquid phase of the effluent nor sorbed to sludge samples. However, within this study no conclusions can be drawn whether Triclosan is removed sorbed to sludge or transformed into other compounds (such as 2,7/2,8-DCDD, Mezcua *et al.* (2004)) or fully metabolised.

Soluble Proteins and Carbohydrates and Extracellular Proteins and Carbohydrates

Soluble protein and carbohydrates revealed casual correlation to Triclosan concentration or removal rates. Soluble proteins and carbohydrates concentration for the 48-hour monitoring lead to the assumption that Triclosan removal within the oxidation ditch might be influenced by available substrates from the inflowing wastewater. However, due to the given varying influences, no specific prediction can be made.

EPS components may be contributing to the hydrophobic and surface charge properties of microbial flocs (Liao *et al.*, 2001; Jorand *et al.*, 1998) and therefore providing possible binding sites to the hydrophobic form of Triclosan. Within the 48-hour monitoring study the extracellular protein and carbohydrate concentrations were found to change to increasing Triclosan concentrations of the bulk phase. However, the weak correlations between the proportions of EPS components and the Triclosan removal were not meaningful enough and require more fundamental information about the variation of boundary conditions and their possible impact of the make-up of EPS on the removal of Triclosan.

Triclosan extraction from sludge and EPS fraction

This is the first known study about the uptake of Triclosan within the EPS fraction. Therefore it is not possible to include any comparison from literature data.

Sorption processes of biomass and sludge may be predicted according to Ternes *et al.* (2004) (*see Chapter 2.5*). As the results of the laboratory test were not available for this thesis (K_d-values for Triclosan and sludge samples), no conclusions can be made according estimated sorption of Triclosan to sludge and the determined Triclosan concentration within the EPS fraction.

However, EPS extraction revealed considerable amounts of sorbed Triclosan and as it has been previously discussed, Triclosan bound to EPS fraction of the suspended solids seemed to adversely effect effluent quality, increasing Triclosan concentrations due to probably re-balancing effects.

The uptake of Triclosan within the EPS seemed to depend on various factors, such as specific oxygen demand within the bulk phase of the oxidation ditch and the particle size, specific surface respectively. Suspended Solids of bigger particle size seemed to enhance Triclosan uptake within the EPS and, once in the effluent, the release of Triclosan to the liquid phase resulting in higher Triclosan effluent concentrations.

Nevertheless, sludge extraction tests for Triclosan have to be improved in order to yield reliable results without interfering compounds and to allow any conclusion about the amount of sorbed Triclosan. This might be achieved by slight changes such as adding an additional clean-up step. Reliable sludge extraction could facilitate the gathering of information on the proportion of sorbed Triclosan to either EPS or cells. Meanwhile the EPS extraction has to be tested for possible cell lysis, especially for fixed biofilms. Degradation products should be included in the determination methods, especially of 2,7/2,8 dibenzo-*p*-dioxin and its possible further degradation products.

Summarising it should be noted that the found correlations are simplified and there might be different, highly complex conclusions, especially if considering diauxic reactions within communal wastewater treatment processes. This should be, in fact, examined within laboratory tests, where boundary conditions are easier to be monitored and controlled.

7 Conclusions and Future Work

- Triclosan has been detected in almost every liquid sample of the wastewater treatment in concentrations up to 5,000 ngL^{-1} for influents and 800 ngL^{-1} for effluents. As there was no method for the determination of degradation products of Triclosan applicable, all discussions are based solely on the observed disappearance of Triclosan and no conclusion can be drawn from either biodegradation or sorption, except for the Triclosan contents extracted from the EPS fraction.

- Tetracycline could not be detected in any of the wastewater treatment plants, nor its degradation products, which might be suggested to their chelating nature with divalent cations, such as calcium and metals, and probably also to prescription patterns.

- Triclosan was shown to be removed in high quantities in all four examined wastewater treatment plants at a rate of up to 96%. Though it is in principle problematic to compare elimination rates for different wastewater treatment plants as their influent conditions may often show wide variations, loss rates for Triclosan within the four different treatment systems varied between 81% and 96%, showing the best and most consistent elimination rate for the oxidation ditch, whereas the lowest average removal rate has been found for the rotating biological contactor (81%).

- Although Triclosan removal rates are shown to be significant high, discharges still contained substantial residual concentrations, which would therefore require further elimination steps, especially if considering the possible formation of 2,7/2,8 dibenzo-*p*-dioxin.

- Primary settling tanks seemed to have high impact on the removal of Triclosan (up to 58% overall removal of Triclosan input to wastewater treatment work), also according to Siegrist *et al.* (2003), who states that sorption coefficients depends on the make-up of the biomass and the physico-chemical properties of the compound. Hydrophobic compounds are more absorbed to primary sludge due to its lipid content, whereas compounds with positively charged groups are adsorbed by negatively charged surfaces of the microorganisms. Enhancing the hydraulic retention time within the primary clarifier might increase the removal capacities within the primary treatment stage.

- All examined biological treatment stages showed significant removal capacities, as follows:
  - Rotating biological contactor - 55.8%
  - Trickling Filter - 39%
  - Oxidation Ditch - 92% (due to high SRT and missing primary settler)
  - Activated Sludge Plant - 36.6%

  Inreasing removal, with regards to the known properties of Triclosan, is only possible through either sorption or biodegradation. Biodegradation might be controlled by optimised conditions or specialised microorganisms, but in general it is difficult to control, especially such parameters which depend on varying influent conditions (COD, TOC, Proteins, Carbohydrates). However, one crucial parameter for higher biodegradation is the solid retention time, which is possible to be alternated in conventional activated sludge plants.

- Triclosan amounts bound in extracellular polymeric substances appear to adversely affect discharge values. This is most likely due to the desorption processes occuring in the secondary clarifier. Hence, Final Clarifier, Polishing Lagoon and Tertiary Reed Bed seemed to have little, if even negative impact on Triclosan overall removal.

  Regarding the possible release of Triclosan from the EPS of sludge flocs within the secondary clarifier, Clara *et al.* (2004) stated better retaining rates for MBR system effluents due to the absence of suspended solids.

- The correlation between liquid and biomass characteristics range from weak to moderate, probably owing to the influences of uncontrolled factors associated with the operation of a full scale treatment work system from which all the samples were obtained.

  Among all of the determined liquid and biomass characteristics temperature, pH and lipid content showed themselves to be the most significant, with regards to the overall removal of Triclosan.

Future works should include

- Lab scale experiments to determined the possible impact of the boundary conditions
- Further investigations of the impact of nutrients on biomass make-up and its possible alteration of sorption or degradation
- Degradation products should be included in the determination methods for both liquid and solid phase. 2,7/2,8 dibenzo-*p*-dioxin should be of special interest, their possible further degradation products and their environmental behaviour.

Further treatment processes, such as the use of activated carbon, membranes, oxidative treatment, sand-filtration, for example, should be investigated. These further treatment steps should also be tested for other environmentally hazardous compounds, regarding the vast array of PPCP substance entering wastewater treatment plants.

Ozonation has been reported to be an effective tool to remove pharmaceuticals (Ternes *et al.*, 2003, 2004), but might pose economic and technical problems to such small wastewater treatment plants. Wastewater chlorination, a commonly used final treatment step, especially in the United States, should be investigated for interactions with pharmaceutical residues. Triclosan has been reported to form a number of chlorinated phenoxy intermediates as chlorination products within drinking water treatment (Vikesland *et al.*, 2003). These species might react further to produce chlorinated phenols and the trihalomethane chloroform. Possible reactions might occur as well for chlorinated wastewater effluents and lead to the release of more toxic by-products into the environment.

Overall it can be concluded that retaining mechanisms for PPCP, Triclosan in particular, might be enhanced by simple alterations of the wastewater treatment process, such as sludge age, but seem nevertheless very complex and further basic investigations are needed. However, it should always be borne in mind that there will not be a 'one- way-solution' due to the vast array of compounds with different properties. Therefore requiring the implementation of multi-barrier system in order to reduce the emission of micropollutants into the aquatic environment.

References

301C O and 301C (1993). Organization for Economic Cooperation and Development, *OECD Guidelines for the Testing of Chemicals* .

Abbt-Braun G and Frimmel F (1991). Spektroskopische Strukturaufklärung aquatischer Huminstoffe, *Vom Wasser* **77**, 291–302.

Adolfsson-Erici M, Petterson M, Parkkonen J and Sturve J (2002). Triclosan, a commonly used bactericide found in human milk and in aquatic environment in Sweden, *Chemosphere* **46**, 1485–1489.

Agüera A, Fernández-Alba A, Piedra L, Mézcua M and Gómez M (2003). Evaluation of triclosan and biphenylol in marine sediments and urban wastewaters by pressurized liquid extraction and solid phase extraction followed by gas chromatography mass spectrometry and liquid chromatography mass spectrometry, *Analytica Chimica Acta* **480**, 193–205.

Al-Doori Z, Morrison D, Edwards G and Gemmell C (2003). Susceptibility of MRSA to triclosan, *Journal of Antimicrobial Chemotherapy* **51**, 185–186.

Alder A, McArdell C, Golet E, Ibric S, Molnar E, Nipales N and Giger W (2001). Occurrence and fate of fluoroquinole, macrolide, and sulfonamide antibiotics during wastewater treatment and in ambient waters in Switzerland, *Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues* pp. 56–69.

Andreozzi R, Raffaele M and Nicklas P (2003). Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment, *Chemosphere* **50**, 1319–1330.

Antusch E (1999), Lokalisierung organischer Schadstoffemissionen in kommunale Abwasserkanäle durch Sielhautuntersuchungen, PhD thesis, Universität Fridericiana zu Karlsruhe (TH), Karlsruhe.

APHA, ed. (1998). *Alpha Standard Methods for Examination of Water and Wastewater*, 20 edn, American Public Health Association.

Austin D, Kristinsson K and Anderson R (1999). The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance, *Proc. Natl. Acad. Science USA* **96**, 1152–1156.

Ayscough N, Farwell J, Franklin G and Young W (2000). Review of humna pharmaceuticals in the environment, *Environmental Agency Bristol England* **P390**.

Baguer A, Jensen J and Krogh P (2000). Effects of the antibiotics oxytetracycline and tylosin on soil fauna, *Chemosphere* **40**, 751–757.

Baker W A and Brown P M (1966). Co(II) and Ni(II) Complexes with Tetracyclines, *Journal of the American Chemical Society* **88**(6), 1314–1317.

Balmer M, Poigner T, Droz C, Romanin K, Bergqvist P A, Müller M and Buser H R (2004). Occurrence of Methyl Triclosan, a Transformation Product of the Bactericide Triclosan, in Fish from Various Lakes in Switzerland, *Environmental Science and Technology* **38**, 390–395.

Baughman G and Paris D (1981). Microbial bioconcentration of organic pollutants from aquatic systems - a critical review, *Critical Review of Microbiology* **8**, 205–228.

Bell J, Elliott G and Smith D (1983). Influence of sewage treatment and urbanization on selection of multiple resistance in fecal coliform populations, *Applied Environmental Microbiology* **46**, 227–232.

Berger K, Petersen B and Buening-Pfaue H (1986). Persistence of drugs occurring in liquid manner in the food chain, *Archiv fuer Lebensmittelhygiene* **37**, 99–102.

Bester K (2003). Triclosan in sewage treatment process - balances and monitoring data, *Water Research* **37**, 3891–3896.

Bester K (2005). Fate of Triclosan and Triclosan-Methyl in Sewage Treatment Plants and Surface Waters, *Archives of Environmental Contamination and Toxicology* **49**, 9–17.

Blankenhorn I and Hornung C (1992). Richtlinie zur Bestimmung von polyzyklischen aromatischen Kohlenwasserstoffen (PAK) in Boden-, Abfall- und Altlastproben, *Landesanstalt für Umweltschutz Baden-Würrtemberg* **LfU-PAK 7/92**, 1–8.

Bouwer E (1989). Transformation of Xenobiotics in Biofilms, *in* W Characklis and P Wilderer, eds, *Structure and Function of Biofilms*, John Wiley and Sons, Inc., New York, USA, pp. 251–267.

Boxall A, Fogg L, Kay P, Blackwell P, Pemberton E and Croxford A (2003). Prioritisation of veterinary medicines in the UK environment, *Toxicology Letters* **142**, 207–218.

Boyd G, Palmeri J, Zhang S and Grimm D (2004). Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and Bayou St. John in New Orleans, Louisiana, USA, *Science of the Total Environment* **333**(1-3), 137–148.

Boyd G, Reemtsma H, Grimm D and Mitra S (2003). Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada, *Science of the Total Environment* **311**, 134–149.

Bradford M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analytical Biochemistry* **72**, 248–254.

Braoudaki M and Hilton A (2004*a*). Adaptive resistance to biocides in Salmonella entericae and Escherichia coli O157 and cross-resistance to antimicrobial agents, *Journal of Clinical Microbiology* **42**, 20–22.

Braoudaki M and Hilton A (2004*b*). Low level of cross-resistance between triclosan and antibiotics in Escherichia coli K-12 and E. coli O55 compared to E. coli O157, *FEMS Microbiology Letter* **235**, 305–309.

Bruss J, Nielsen K and Keiding K (1992). On the stability of activated sludge flocs with implications to dewatering, *Water Science and Technology* **26**(12), 1597–1604.

Buerge I, Poigner T, Müller M and Buser H (2003). Caffeine, an anthropogenic marker for wastewater contamination of surface waters, *Environmental Science and Technology* **37**, 691–700.

Campagnole M, Bourgeois M and Mountaudon E (2002). Fonctionnalisation des gamma- et delat-pyronène, Synthèse et étude de la réactivité des composés peroxydiques, *Tetrahedron* **58**(6), 1165–1171.

Characklis W and Marshall K (1990). Biofilms: A basis for an interdisciplinary approach, *in* W Characklis and K Marshall, eds, *Biofilms*, John Wiley and Sons, Inc., New York, USA, pp. 3–17.

Christensen B and Characklis W (1990). Physical and chemical properties of biofilms, *in* W Characklis and K Marshall, eds, *Biofilms*, John Wiley and Sons, Inc., New York, USA, pp. 93–130.

Christian T, Schneider R, Färber H, Skutlarek D, Meyer M and Goldbach H (2003). Determination of antibiotic residues in manure, soil, and surface waters, *Acta hydrochimica and Hydrobiologica* **31**(1), 36–44.

Chuanchuen R, Beinlich K, Hoand T, Becher A, Karkhoff-Schweizer R and Schweizer H (2001). Cross-resistance between triclosan and antibiotics in Pseudomonas aeruginosa is mediated by multidrug efflux pump: Exposure of a suspectible mutant strain to triclosan selects nfx mutants overexpressing MexCD-OprJ, *Journal of Antimicrobial Agents and Chemotherapy* **45**, 428–432.

Ciba Speciality Chemicals (2001*a*). Irgasan DP 300, Irgacare MP. General information on chemical, physical and microbiological properties, (Technical Brochure 2520). AgB2520e.02.2001.

Ciba Speciality Chemicals (2001*b*). Irgasan DP 300, Irgacare MP. Toxicology and ecological data, (Technical Brochure 2521).

Ciba Specialty Chemical (1998). *Facts Sheet - PEC Calculation of Triclosan*, Basel, Switzerland.

Clara M, Strenn B, Ausserleitner M and Kreuzinger N (2004). Comparison of the behavior of selected micropollutants in a membrane bioreactor and a conventional wastewater treatment plant, *Water Science and Technology* **50**(5), 29–36.

Cleuvers M (2003). Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects, *Toxicology Letters* **142**, 185–194.

Colborn T and Clement C (2004). *Chemically induced alterations in sexual and functional development: The wildlife/human connection*, Princetown Scientific Co., New Jersey, USA.

Cole E, Addison R, Rubino J, Leese K, Dulaney P, Newell M, Wilkins J, Graber D, Wineinger T and Criger D (2003). Investigation of antibiotic and antibacterial agent cross-resistance in target bacteria from homes of antibacterial product users and nonusers, *Journal of Applied Microbiology* **95**, 664–676.

Daughton C and Ternes T (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change?, *Environmental Health Perspectives* **107**(6), 907–938.

de Groot A, Weyland J and Nater J (1994). *Unwanted effects of cosmetics and drugs used in dermatology*, 3 edn, Elsevier, London, New York, Tokyo.

Diaz-Cruz M S, Alda M and Barceló D (2003). Environmental behaviour and analysis of veterinary and human drugs in soils, sediments and sludge, *Trends in Analytical Chemistry* **22**(6), 340–351.

Dignac M, Ginestet P, Ryback D, Bruchet A, Urbain V and Scribe P (2000). Fate of wastewater organic pollution during activated sludge treatment: nature of residual organic matter, *Water Research* **34**(17), 4185–4194.

Dokianakis S, Kornaros M and Lyberatos G (2004). On the effect of pharmaceuticals on bacterial nitrite oxidation, *Water Science and Technology* **50**(5), 341–346.

dos Santos H, de Almeida W and Zerner M (1997). Conformational analysis of the anhydrotetracycline molecule: a toxic decomposition product of tetracycline, *Journal of Pharmaceutical Science* **87**(2), 190–195.

Dubois M, Gilles K, Hamilton J, Rebers P and Smith F (1956). Colorimetric method for determination of sugars and related substances, *Analytical Chemistry* **28**(3), 350–356.

EN 12879 (2000). Characterisation of sludges - Determination of the loss on ignition of dry mass, Technical report, CEN.

EN 12880 (2000). Characterisation of sludges - Determination of dry residue and water content, Technical report, CEN.

Endtz H, van Kungeren G, Jansen W, van der Reyden T and Mouton R (1991). Quinoloe resistance in Campylobacter isolated from man and poultry following the introduction of fluoroquinoles in veterinary medicine, *Antimicrobial Agents and Chemotherapy* **27**, 199–208.

Eriksson L and Alm B (1991). Study of flocculation mechanisms by observing effects of a complexing agent on activated sludge properties, *Water Science and Technology* **24**(7), 21–28.

Färber H and Skutlarek D (2004). *Vorkommen und Abbau von Antibiotika in Trink- und Abwässern*, Presentation at *Kooperationsforum Innovation: Arzneimittelrückstände und endokrin wirksame Stoffe in Trink- und Abwasser* - WEDECO AG, Mülheim/Ruhr. 30. 03. 2004.

Federle T, Kaiser S and Nuck B (2002). Fate and effects of Triclosan in activated sludge, *Environmental Toxicology and Chemistry* **21**(7), 1330–1337.

Flemming H C (1995). Sorption sites in biofilms, *Water Sciences and Technology* **32**(8), 27–33.

Foran C, Bennett E and Benson W (2000). Development evaluation of a potential non-steroidal estrogen: triclosan, *Marine Environmental Research* **50**, 153–156.

Forth W, Henschler D, Rummel W and Starke K (1992). *Allgemeine und spezielle Pharmakologie und Toxikologie*, 6 edn, BI-Wissenschaftsverlag.

Fraker S and Smith G (2004). Direct and interactive effects of ecologically relevant concentration of organic wastewater contaminations on Rana pipiens Tadpoles, *Environmental Toxicology* **19**, 250–256.

Franklin T and Snow G, eds (1998). *Biochemistry of Antimicrobial Action*, 4 edn, Chappman and Hall, London.

Friege H, Buysch H, Leuchs W, Hembrock A and König W (1989). Belastung von Klärschlämmen und Böden mit organischen Schadstoffen, *Korrespondenz Abwasser* **36**, 601–608.

Frølund B, Palmgren R, Keiding K and Nielsen P (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin, *Water Research* **30**, 1749–1758.

Garrison A, Pope J and Allen F (1976). Identification and Analysis of Organic Pollutants in Water, *Ann Arbor Science* **MI**, 517–566.

Giger W, Alder A, Golet E, Kohler H P, McArdell C, Moldnar E, Siegrist H and Suter M F (2003). Occurrence and fate of antibiotics as trace contaminants in wastewaters. sewage sludges, and surface waters, *Chimica* **57**(9), 485–491.

Glaeske G (1998). Arzneimittel in Gewässern - Risiko für Mensch, tier und Umwelt? - Konsequenzen unter Berüchsichtigung des Arzneimittelverbrauchs, *Hessische Landesanstalt für Umweltschutz, HLFU Schriftenreihe Umweltplanung, Arbeits- und Umweltschutz* **254**, 97–104.

Golet E, Alder A, Hartmann A, Ternes T and Giger W (2001). Trace determination of fluoroquinole antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection, *Analytical Chemistry* **73**(15), 3632–3638.

Goni-Urriza M, Capdepuy M, Arpin C, Raymond N, Caumette P and Quentin C (2000). Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and Aeromonas spp., *Apllied and Environmental Microbiology* **66**(1), 125–132.

Goodwin J and Foster C (1985). A further examination into the composition of activated sludge surfaces in relation to their settlement characteristics, *Water Research* **19**, 527–533.

Gremm T and Kaplan L (1997). Dissolved carbohydrates in streamwater determined by HPLC and pulsed amperiometric detection, *Limnology and Oceanography* **42**, 385–393.

Guardabassi L, Wong D L F and Dalsgaard A (2002). The effect of tertiary wastewater treatment on the prevalence of antimicrobial resistance, *Water Research* **36**, 1955–1964.

Halling-Sørensen B (2001). Inhibition of aerobic growth system and nitrification of bacteria in sewage sludge by antibacterial agents, *Archives of Environmental Contamination and Toxicology* **40**, 451–460.

Halling-Sørensen B, Nielsen N, Lansky P, Ingerslev F, Lützhøft H H and Jørgensen S (1998). Occurrence, fate and effects of pharmaceutical substances in the environment - a review, *Chemosphere* **36**, 357–394.

Halling-Sørensen B, Sengelov G and Tjørnelund J (2002). Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria, *Environmental Contamination and Toxicology* **42**, 263–271.

Hamscher G, Pawelzick H, Höper H and Nau H (2005). Different behaviour of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manner, *Environmental Toxicology and Chemistry* **24**, 861–868.

Hamscher G, Sczesny S, Höper H and Nau H (2002). Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry, *Analytical Chemistry* **74**, 1509–1518.

Hartmann A, Alder A, Koller T and Widmer R (1998). Identification of fluoroquinolone antibiotics as the main source of umuC genotoxicity in native hospital wastewater, *Environmental Toxicology and Chemistry* **17**, 377–382.

Hay A, Dees P and Sayler G (2001). Growth of a bacterial consortium on triclosan, *FEMS Microbiology Ecology* **36**, 105–112.

Heath R and Rock C (2000). A triclosan-resistant bacterial enzyme, *Nature* **406**, 145–146.

Heberer T (2002). Tracking persistent pharmaceutical residues from municipal sewage to drinking water, *Journal of Hydrology* **266**, 175–189.

Heberer T, Dünnbier U, Reilich C and Stan H J (1997). Detection of drugs and drug metabolites in ground water samples of a drinking water treatment plant, *Fresenius Environmental Bulletin* **6**, 438–443.

Heberer T, Feldmann D, Reddersen K, Altmann H J and Zimmermann T (2002). Production of drinking water from highly contaminated surface waters: removal of organic, inorganic , and microbial contaminants applying mobile membrane filtration units, *Acta hydrochimica hydrobiologica* **30**, 24–33.

Hirsch R, Ternes T, Haberer K and Kratz K (1999). Occurrence of antibiotics in the aquatic environment, *The Science of the Total Environment* **225**, 109–118.

Holm J, Rügge K, Bjerg P and Christensen T (1995). Occurrence and distribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill, *Environmental Science and Technology* **29**, 1415–1420.

Huber S (1999). *Einfluss der Prozessführung auf Menge und Zusammensetzung von Proteinen und Polysacchariden im Ablauf von Sequencing-Batch-Reaktoren*, Vol. 152, Berichte aus der Wassergüte- und Abfallwirtschaft, Technische Universität München.

Hundt K, Martin D, Hammer E, Jonas U, Kindermann M and Schauer F (2000). Transformation of Triclosan by Trametes versicolor and Pycnoporus cinnabarinus, *Applied and Environmental Microbiology* **66**(9), 4157–4160.

Ishibashi H, Matsumura N, Hirano M, Matsuoka M, Shiratsuchi H, Tako Y and Arizono K (2003). Effects of triclosan on the early life stages and reproduction of medaka Oryzias latipes and induction of hepatic vitellogenin, *Aquatic Toxicology* **67**, 167–179.

Iwane T, Urase T and Yamamoto K (2001). Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water, *Water Science and Technology* **43**(2), 91–99.

Jacobsen B, Arvin E and Reinders M (1996). Factors affecting sorption of pentachlorphenol to suspended microbiomass, *Water Resources* **30**, 13–20.

Jobling S, Sheahan D, Osborne J, Matthiesen P and Sumpter J (1996). Inhibition of testicular growth in rainbow trout exposed to estrogenic alkylphenolic chemicals, *Environmental Toxicology and Chemistry* **15**, 194–202.

Jones R, Jampani H, Newman J and Lee A (2000). A review of effectiveness and safety in health care settings, *American Journal of Infection Control* **28**, 184–196.

Jorand F, Boué-Bigne F, Block J and Urbain V (1998). Hydrophobic-Hydrophilic properties of activated sludge exopolymeric substances, *Water Science and Technology* **37**(4-5), 307–315.

Jungermann E (1968). Soap bacteriostats, *Journal of American Oil Chemistry Society* **45**, 345–350.

Kanda R, Griffin P, James J and Fothergill J (2003). Pharmaceutical and personal care products in sewage treatment works, *Journal of Environmental Monitoring* **5**, 823–830.

Kanetoshi A, Ogawa H, Katsura E, Kaneshima H and Miura T (1988*a*). Formation of polychlorinated dibenzo-p-dioxin from 2,4,4'-trichloro-2'-hydroxydiphenyl ether (IRGASAN DP300) and its chlorinated derivatives by exposure to sunlight, *Journal of Chromatography* **454**, 145–155.

Kanetoshi A, Ogawa H, Katsura E, Kaneshima H and Miura T (1988*b*). Formation of polychlorinated dibenzo-p-dioxin upon combustion of commercial textile products containing 2,4,4'-trichloro-2'-hydroxydiphenyl ether Irgasan DP300, *Journal of Chromatography* **442**, 289–299.

Klein N and Cunha B (1995). Third generation cephalosphorins, *Med Clin North Am* **79**, 705–719.

Kolpin D, Furlong E, Meyer M, Thurman E, Zaugg S, Barber L and Buxton H (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: a national reconnaissance, *Environmental Science and Technology* **36**, 1202–1211.

Kuch B, Schneider C and Metzger J (2003). Monitoring of the disinfectants triclosan, triclocarban and hexachlorophene in rivers, sediments, sewage sludge, in- and effluents of wastewater treatment plants, *Forschungsbericht FZKA-BWPLUS, BWB 21009* .

Kümmerer K, Al-Ahmada A and Mersch-Sundermann V (2000). Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test, *Chemosphere* **40**, 701–710.

Lallai A, Mura G and Onnis N (2002). The effects of certain antibiotics on biogas production in the anaerobic digestion on pig waste slurry, *Bioresource Technology* **82**, 205–208.

Laschka D and Trumpp M (1991). Sielhautuntersuchungen zur Lokalisierung von AOX-Emittenten im Kanalnetz, *Korrespondenz Abwasser* **38**, 495–496.

Latch D, Packer J, Arnold W and McNeill K (2003). Photochemical conversion of Triclosan to 2,8-dichlorodibenzo-p-dioxin in aqueous solution, *Journal of Photochemistry and Photobiology A: Chemistry* **158**, 63–66.

Lee T, Kim J and Hwang S (2003). Hydrogel patches containign triclosan for acne treatment, *European Journal of Pharmaceutics and Biopharmaceutics* **56**(3), 407–412.

Levy S, Roujeinikova A, Sedelnikova S, Baker P, Stuitje A, Salbas A, Rice D and Rafferty J (1999). Molecular basis of Triclosan activity, *Nature* **398**, 383–384.

Li D and Ganczarczyk J (1990). Structure of activated slugde flocs, *Biotechnology and Bioengineering* **35**, 57–65.

Liao B, Allen D, Droppo I, Leppard G and Liss S (2001). Surface properties of sludge and their role in bioflocculation and settleability, *Water Research* **35**(2), 339–350.

Lindsey M, Meyer M and Thurman E (2001). Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry, *Analytical Chemistry* **73**(19), 4640–4646.

Lindström A, Buerge I and Poiger T (2002). Occurrence and Environmental Behaviour of the Bactericide Triclosan and its Methyl Derivative in Surface Waters and in Wastewaters, *Environmental Science and Technology* **36**, 2322–2329.

Liu Y, Lam M and Fang H (2001). Adsorption of heavy metals by EPS of activated sludge, *Water Science and Technology* **43**(6), 59–66.

Loke M, Tjørnelund J and Halling-Sørensen B (2002). Determination of the distribution coefficient (logKd) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure, *Chemosphere* **48**, 351–361.

Lowry O, Rosebrough N, Farr A and Randall R (1951). Protein measurement with the Folin phenol reagent, *Journal of Biological Chemistry* **193**, 365–375.

McArdell C, Molnar E, Suter M F and Giger W (2003). Occurrence and fate of macrolide antibiotics in wastewater treatment plants and the Glatt Valley Watershed (Switzerland), *Environmental Science and Technology* **37**(24), 5479–5486.

McArdell C, Clara M, A.Göbel, Joss A, Keller E, Kreuzinger N, Plüss H, Siegrist H and Strenn B (2003). Impact of treatment technologies illustrated with the mass balance on the PPCPs selected in POSEIDON, *in POSEIDON Symposium Presentation of Project Results*, Braunschweig, Germany.

McAvoy D, Schatowitz B, Jacob M, Hauk A and Eckhoff W (2002). Measurement of Triclosan in wastewater treatment systems, *Environmental Toxicology and Chemistry* **21**(7), 1323–1329.

McBain A, Rickard A and Gilbert P (2002). Possible implication of biocide accumulation in the environment on the prevalence of bacterial antibiotic resistance, *Journal of Industrial Microbiology and Biotechnology* **29**, 326–330.

McKinley V and Vestal J (1985). Physical and chemical correlates of microbial activity and biomass in composting municipal sewage sludge, *Applied and Environmental Microbiology* **50**(6), 1395–1403.

McMurry L, Oethinger M and Levy S (1998). Triclosan targets lipid synthesis, *Nature* **394**, 531–532.

Menoutis J and Parisi A (2001). Triclosan and its impurities, *Quantex Laboratories Technology Review* . **URL:** *http://www.quantexlabs.com/triclosan.htm*

Merck E (1974). *Klinisches Labor, Gesamtlipide. I. Photometrische Bestimmung*, 12 edn, Darmstadt.

Merck E (1983). *The Merck Index*, Rahway, NJ, USA.

Mezcua M, Gómez J, Ferrer I, Aguera A, Hernando M and Fernández-Alba A (2004). Evidence of 2,7/2,8-dibenzodichloro-p-dioxin as photodegradation product of triclosan in water and wastewater samples, *Analytica Chimica Acta* **524**, 241–247.

Miao X S, Bishay F, Chen M and Metcalfe C (2004). Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada, *Environmental Science and Technology* **38**, 3533–3541.

Miranda C and Zemelman R (2001). Antibiotic resistant bacteria in fish from the Concepción Bay, Chile, *Marine Pollution Bulletin* **42**(11), 1096–1102.

Miyazaki T, Yamagishi T and Matsumoto M (1984). Residues of 4-chloro-1-(2,4-dichlorodiphenoxy)-2-methoxybenzene (triclosan methyl) in aquatic biota, *Bulletin of Environmental Contamination and Toxicology* **32**, 227–232.

Morgan J, Foster C and Evison L (1990). A comparitive study of the nature of biopolymers extracted from anaerobic and activated sludge, *Water Research* **24**, 743–750.

Morrall D, McAvoy D, Schatowitz B, Inauen J, Jacob M, Hauk A and Eckhoff W (2003). A field study of triclosan loss rates in river water (Cibolo Creek, TX), *Chemosphere* **54**, 653–660.

Neyens E, Baeyens J, Dewil R and de Heyder B (2004). Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering, *Journal of Hazardous Materials* **106B**, 83–92.

Nilsson C, Andersson C and Rappe S (1974). Chromatographic evidence for the formation of chlorodioxins from chloro-2-phenoxyphenols, *Journal of Chromatography* **96**(137-147).

Oaks J, Gilbert M, Virani M, Watson R, Meteyer C, Rideout B, Shivaprasad H, Ahmed S, Chaudhry M, Arshad M, Mahmood S, Ali A and Khan A (2004). Diclofenac residues as the cause of vulture population decline in Pakistan, *Nature* **427**, 596–598.

Ohnishi S and Barr J (1978). A simplified method of quantitating protein using the biuret and phenol reagents, *Analytical Biochemistry* **86**(1), 193–200.

Oka H, Ikai Y, Kawamura N, Yamada M, Harada K and Ito S (1989). Photodecomposition products of Tetracycline in aqueous solutions, *Journal of Agriculture and Food Chemistry* **37**, 226–231.

Oka H, Uno K, Harada K, Yasaka K and Suzuki M (1984). A simple method for the analysis of tetracyclines using reversed-phase high-performance liquid chromatography, *Journal of Chromatography* **298**, 435–443.

Okumura T and Nishikawa Y (1996). Gas chromatography-mass spectrometry determination of Triclosan in water, sediment and fish samples via methylation with diazomethane, *Analytica Chimica Acta* **325**, 175–184.

Onken D (1985). *Anitbiotika - Chemie und Anwendung*, 2 edn, Akademie-Verlag Berlin.

Orvos D, Versteeg D, Inauen J, Capdevielle M, Rothenstein A and Cunningham V (2002). Aquatic toxicity of Triclosan, *Environmental Toxicology and Chemistry* **21**(7), 1338–1349.

Paxéus N (2004). Removal of selected non-steroidal anti-inflammatory drugs (NSAIDs), gemfibrozil, carbamazepine, ß-blockers, trimethoprim and triclosan in conventional wastewater treatment plants in five EU countries and their discharge to the aquatic environment, *Water Science and Technology* **50**(5), 253–260.

Pedersen B and Nielsen U (2003). Monitoring programme for wastewater treatment plant Lynetten, household chemicals and endocrine disrupting substances, *Report to Lynettefaellesskabet I/S* .

Peterson G (1977). A simplification of the protein assay method of Lowry et al. which is more generally applicable, *Analytical Biochemistry* **83**(2), 346–356.

Purdom C, Hardiman P, Bye V, Eno N and Sumpter C T J (1994). Estrogenic effects of effluents from sewage treatment works, *Chemical Ecology* **8**, 275–285.

Qiting J and Xiheng Z (1988). Combination process of anaerobic digestion and ozonation technology for treating wastewater from antibiotic production, *Water treatment* **3**, 285–291.

Reinthaler F, Posch J, Wüst G, Haas D, Ruckenbauer G, Mascher F and Marth E (2003). Antibiotic resistance of E. coli in sewage and sludge, *Water Research* **37**, 1685–1690.

Reverté S, Borrull F, Pocurull E and Marcé R (2003). Determination of antibiotic compounds in water by solid-phase extraction-high-performance liquid chromatography-(electrospray) mass spectrometry, *Journal of Chromatography A* **1010**, 225–232.

Routledge E, Sheahan D, Desbrow C, Brighty G, Waldock M and Sumpter J (1998). Identification of estrogenic chemicals in STW effluents, *Environmental Science and Technology* **8**, 275–285.

Sabaliunas D, Webb S, Hauk A, Jacob M and Eckhoff W (2003). Environmental fate of Triclosan in the River Aire Baisin, UK, *Water Research* **37**, 3145–3154.

Sacher F, Lange F, Brauch H and Blankenhorn L (2001). Pharmaceuticals in groundwaters - analytical methods and results of a monitoring program in Baden-Wuerttemberg, Germany, *Journal of Chromatography A* **938**(1-2), 199–210.

Samuelsen O, Torsvik V and Ervik A (1992). Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after medication, *Science of the Total Environment* **114**, 25–36.

Sanz J, Rodríguez N and Amils R (1996). The action of antibiotics on the anaerobic digestion process, *Applied Microbiology and Biotechnology* **46**, 587–592.

Schettgen C (2000), Bioakkumulation bei verschiedenen pH-Werten des Wassers und der Pyrethroide Cyfluthrin, Cypermethrin, Deltamethrin und Permethrin, PhD thesis, Universität Oldenburg, Oldenburg.

Schmitt J, Nivens D, White D and Flemming H C (1995). Change of biofilm properties in response to sorbed substances - an FTIR-ATR study, *Water Science and Technology* **32**(8), 149–155.

Schneider C, Kuch B, Hohlstein C, Gaiser S and Metzger J (2004*a*). Fate of synthetic organic substances (SOS) in wastewater treatment process - Part II: Ibuprofen and Triclosan, *in 14th Annual Meeting of SETAC Europe*, Prague, Czech Republic.

Schneider C, Kuch B, Hohlstein S, Gaiser J and Metzger J (2004*b*). Fate of synthetic organic substances (SOS) in wastewater treatment process - Part IV: Conclusion and Assessment, *in 14th Annual Meeting of SETAC Europe*, Prague, Czech Republic.

Schweitzer H P (2001). Triclosan: a widely used biocide and its links to antibiotics, *FEMS Microbiology Letters* **202**, 1–7.

Sczesny S, Nau H and Hamscher G (2003). Residue Analysis of Tetracyclines and Their Metabolites in Eggs and in the Environment by HPLC Coupled with a Microbiological Assay and Tandem Mass Spectrometry, *Journal of Agricultural and Food Chemistry* **51**, 697–703.

Siegrist H, Joss A, Alder A, McArdell-Bürgisser C, Göbel A, Keller E and Ternes T (2003). Micropollutants - new challenge in wastewater disposal?, *EAWAG News* **57**, 7–10.

Siegrist H, Joss A, Alder A, Golet E, Gobel A, Keller E, McArdell C and Kreuzinger K (2003). The fate and removal of pharmaceuticals during sewage treatment, *in POSEIDON Symposium Presentation of Project Results*, Braunschweig, Germany.

Singer H, Müller S, Tixier C and Pillonel L (2002). Triclosan: Occurrence and fate of a widely used biocide in the aquatic environment: Field measurements in Wastewater Treatment Plants, Surface Waters, and lake Sediments, *Environmental Science and Technology* **36**, 4998–5004.

Skoog D, West D, Holler F and Crouch S (2000). *Analytical Chemistry - An introduction*, 7 edn, Saunders College Publishing, Fort Worth.

Späth R, Flemming H C and Wuertz S (1998). Sorption properties of biofilms, *Water Science and Technology* **37**(4-5), 207–210.

Speer B, Shoemaker N and Salyers A (1992). Bacterial resistance to tetracycline: mechanism, transfer and clinical significance, *Clin Microbiol Rev* **5**(4), 387–399.

Stan H J and Heberer T (1997). Determination of clofibric aid and N-(Phenylsulfonyl)-Sarcosine in sewage, river and drinking water, *Environmental Analytical Chemistry* **67**, 113–134.

Stan H J, Heberer T and Schmidt-Bäumler K (1999). Occurrence and distribution of organic contaminants in the aquatic system in Berlin. Part II: Substituted Phenols in Berlin surface water, *Acta Hydrochimica and Hydrobiologica* **27**(3).

Stumpf M, Ternes T, Haberer K, Seel P and Baumann W (1996). Nachweis von Arzneimittelrüständen in Kläranlagen und Fliessgewässern, *Vom Wasser* **86**, 291–303.

Ternes T, Loeffler D, Knacker T, Alder A, Joss A and Siegrist H (2003). Sorption onto sludge from municipal STPs: A relevant process for removal of pharmaceuticals and musk fragrances, *in 228th American Chemical Society meeting*, Philadelphia, USA.

Ternes T, Stumpf M, Müller J, Haberer K, Wilken R and Servos M (1999). Behavior and occurrence of estrogens in municipal sewage treatment plants - I. Investigations in Germany, Canada and Brazil, *Science of the Total Environment* **225**, 81–90.

Ternes T (1998). Occurrence of drugs in German sewage treatment plants and rivers, *Water Research* **31**(11), 3245–3260.

Ternes T (2001). Pharmaceuticals and metabolites as contaminants of the aquatic environment, *Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues. Symposium Series 791, American Chemical Society, Washington DC* pp. 39–54.

Ternes T, Hirsch R, Stumpf M, Eggert T, Schuppert B and Haberer K (1999). Nachweis und Screening von Arzneimittelrückständen, Diagnostika und Antiseptika in der aquatischen Umwelt, *Abschlussbericht zum BMBF-Forschungsvorhaben 02WU9667/3 - ESWE-Institut für Wasserforschung und Wassertechnologie GmbH, Mainz* .

Ternes T, Janex-Habibi M L, Knacker T, Kreuzinger N and Siegrist H (2004). Assessment of Technologies for the removal of pharmaceuticals and personal care products in sewage and drinking water facilities to improve the indirect potable water reuse, *POSEIDON, Detailed report related to the overall project duration (1.1.2001-30.6.2004)* **EVK1-CT-2000-00047**.

Ternes T, Ried A, Herrmann N, Kampmann M, Teiser B, Hüber M and von Gunten U (2003). Effluent Ozonation : a promising upgrade for micropollutant removal in municipal wastewater treatment, *in POSEIDON Symposium Presentation of Project Results*, Braunschweig, Germany.

Thiele-Bruhn S (2003). Pharmaceutical antibiotic compounds in soils - a review, *Journal of Plant Nutrial Soil Science* **166**, 145–167.

Thiele S and Beck I (2001). Wirkungen pharmazeutischer Antibiotika auf die Mikroflora - Bestimmung mittels ausgewählter bodenbiologischer Testverfahren, *Mitteilungen der deutschen bodenkundlichen Gesellschaft* **96**, 383–384.

Thomas P and Foster G (2005). Tracking acidic pharmaceuticals, caffeine, and triclosan through the wastewater treatment process, *Environmental Toxicology and Chemistry* **24**(1), 25–30.

Thompson A, Winkler G, Griffin P, Stuetz R and Cartmell E (2004). The fate and removal of pharmaceuticals during sewage treatment, *in Proceedings of the 16th International Congress of Chemical and Process Engineering*, Praha, Czech Republic.

Tolls J (2001). Sorption of veterinary pharmaceuticals in soils: a review, *Environmental Science and Technology* **35**(17), 3397–3406.

Tsezos M and Bell J (1991). Comparison of the biosorption and desorption of hazardous organic pollutants by live and dead biomass, *Water Research* **23**, 561–568.

Umweltbundesamt Wien (1995). *Analytische Untersuchung von Klärschlamm - Analysenbericht des Umweltbundesamtes Nr. UBA-BE-046*, Bundesministerium für Umwelt, Wien, Österreich.

Urbain V, Block J and Manem J (1993). Bioflocculation in activated sludge: an analytical approach, *Water Research* **27**, 829–838.

van der Heide E and van de Plas E H (1984). Geneesmiddelen en milieu, *Pharmaceutisch Weekblad* **119**, 936–1647.

Vartanian V, Goolsby B and Brodbelt J (1998). Identification of tetracycline antibiotics by electrospray ionisation in a quadrupole ion tap, *Journal of American Society for mass Spectrometry* **9**, 1089–1098.

Vikesland P, Rule K and Greyshock A (2003). Triclosan fate in chlorinated and chloraminated waters, *in 228th American Chemical Society meeting*, Philadelphia, USA.

Webb S, Ternes T, Gibert M and Olejniczak K (2003). Indirect human exposure to pharmaceuticals via drinking water, *Toxicology Letters* **142**, 157–167.

Weber W, McGinley P and Katz L (1991). Sorption phenomena in subsurface systems: Concepts, models and effects on contaminant face and transport, *Water Research* **25**, 499–528.

Wegener H, Aarestrup F, Gerner-Smidt P and Bager F (1990). Transfer of antibiotic resistant bacteria from animals to men, *Acta of Veterinary Scandinavian Suppl.* **92**(51).

Wilson B, Smith V, jr. F D and Larive C (2003). Effects of three pharmaceutical and personal care products on natural freshwater algae assemblages, *Water Science and Technology* **37**, 1713–1719.

Winckler C and Graffe R (2000). Characterisation and utilisation of waters from intensive animal production with regard to soil, *Chemosphere* **40**, 759–765.

Wingender J, Neu T and Flemming H C (1999). What are Bacterial Extracellular Polymeric Substances, *in* J Wingender, T Neu and H C Flemming, eds, *Microbial Extracellular Polymeric Substances - Characterization, Structure and Function*, Springer, Berlin, Germany.

Witte H, T.Langenohl and Offenbächer G (1989). Untersuchung zum Eintrag von organischen Schadstoffen in Boden und Pflanzen durch landwirtschaftliche Klärschlammverwertung, *Korrespondenz Abwasser* **35**, 440–448.

Wollenberger L, Halling-Sørensen B and Kusk K (2000). Acute and chronic toxicity of veterinary antibioitcs to Daphnia magna, *Chemosphere* **40**, 723–730.

Wuertz S, Pfleiderer P, Kriebitzsch K, Späth R, Griebe T, Coello-Oviedo D, Wilderer P and Flemming H C (1998). Extracellular redox activity in activated sludge, *Water Science and Technology* **37**(4-5), 379–384.

Yang S and Carlson K (2004). Evolution of antibiotic occurrence in a river through pristine urban and agricultural landscapes, *Water Research* **37**, 4645–4556.

Young H K (1993). Antimicrobial resistance spread in aquatic environments, *Journal of Antimicrobial Chemotherapy* **31**, 627–635.

Zhang X, Bishop P and Kinkle B (1999). Comparison of extraction methods for quantifying extracellular polymers in biofilms, *Water Science and Technology* **39**(7), 211–218.

Zhu J, Snow D, Cassada D, Monson S and Spalding R (2001). Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid-phase extraction and liquid chromatography - tandem mass spectrometry, *Journal of Chromatograohy A* **928**, 177–186.

Appendix

tetracycline, CAS-Nr.	chemical name and formular [a]	source	molecular weight [a]	pKa			solubility [e]
Chlortetracycline hydrochloride	2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride [4S-(4 alpha, 4a alpha,5a alpha,6 beta,12a alpha)] $C_{22}H_{23}C_1N_2O_{83}\cdot HCl$	Isolated from the fungus Streptomyces aureofaciens [b]	515.34	3,3 [c]	7,4 [c]	9,3 [c]	sparingly soluble in water; soluble in solutions of alkali hydroxides and carbonates; slightly soluble in alcohol; practically insoluble in acetone, in chloroform, in dioxane and in ether
Oxytetracycline hydrochloride 3380-34-5	2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-,monohydrochloride, [4S-(4 alpha,4a alpha,5 alpha,5a alpha,6 beta,12a alpha)] $C_{22}H_{24}N_2O_9\cdot HCl$	Isolated from the fungus Streptomyces rimosus [b]	496.89	3,3 [d]	3,7 [d]	9,1 [d]	freely soluble in water, but crystals of OTC base separate as a result of partial hydrolysis of the hydrochloride. Sparingly soluble in alcohol and in methanol, and even less soluble in dehydrated alcohol; insoluble in chloroform and in ether
Tetracycline hydrochloride 64-75-5	2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-,monohydrochloride, [4S-(4 alpha,4a alpha,5a alpha,6 beta,12a alpha)] $C_{22}H_{24}N_2O_8\cdot HCl$	Produced by some streptomyces strains; however, it is manufactured by hydrogenolysis of chlortetracycline [f]	480.90	8,3 [c]	10,2 [c]		soluble in water and in solutions of alkali hydroxides and carbonates; slightly soluble in alcohol, practically insoluble in chloroform and ether acid

[a] USP dictionary of USAN and international drug names, 2002 ed. Rockville, MD: The United States Pharmaceutical Convention, Inc., 2002
[b] Baggary TB. Veterinary drug therapy. Baltimore: Lea & Febiger, 1994: 264-92
[c] Riviere J., Craigmill A.L., Sundlof S.F., Handbook of comperative pharmacokinetics and residues of veterinary antimicrobials, Boca Raton, FL: CRC Press, Inc, 1991:175-226
[d] Panel comment, Rec 3/5/96
[e] The United States pharmacopeia. The national formulary. USP 26th revision (Jan, 2003). NF 21st ed (Jan, 2003). Rockville, MD: The United States Pharmacopeial Convention, Inc., 2002
[f] Ketterer P.J., Dunster P.J., Failure to eliminate Leptospira pomona from pigs by treatment with long acting oxytetracycline. Aus Vet J 1985 Oct; 62(10):348-9

Appendix 4.2.5 :

Sampling Stratification for Full Scale Watewater Treatment Plants

1. Introduction

This sampling stratification should be used as an advice for Samples of Full Scale Wastewater Treatment Plants (WTP's). It aims a regular sample collection between the selected sampling sites for providing a steady and comprehensible withdrawal of samples.

It will deal with the type of samples which will have to be taken, the sampling technique for each STP, the sample preservation, preparation, storage and as appendix a sampling protocol to be filled out by each sampling.

2. Sampling Site

All sampling sites have been chosen because of their predicted detectable load of some selected pharmaceuticals. The Sampling of each WTP aims to produce an overview of the fate and occurrence of those selected pharmaceuticals.

As all interactions in a full scale sewage treatment plant depend on lots of influences, it will be necessary to obtain indispensable information about each sampling site.

The following listing will give an overview of possible notes (which can be verified in due course):

- Type of Sewage treatment Plant and sewer system
- Connected Population / population equivalent
- Amount and kind of industrial influent
- average inflow (Q/d)
- maximal inflow (Q_{max}/h)
- dry weather inflow (Q_{dry}/h)
- design inflow/design population
- inflow and outflow rates/datas
- max/min/average hydraulic retention time
- max/min/average solid retention time
- amount of return flow sludge
- ratio *primary:secondary:tertiary* sludge (*retention time, amount, treatment, waste disposal)*
- amount of disposal sludge per pop equivalent *or/and* per month *or/and* per m³ inflow

3. Sampling Technique – Sampling Preservation

Initially random samples will be taken for all sampling sites. Composite sampling will be necessary for gaining reliable data, which should be done by mixing 24-hour samples taken by automatic samplers. It will be figured out a conformist sampling protocol for each WTP. To be sure that every sampling can be comprehensive those protocols shall be regarded and filled out whenever a sample is taken, the sample number mentioned in the protocol shall also appear on the sample container together with location, date, hour, name of sampler and -if used- added preservation chemical. In advance must also be checked up what measurements have to be made to not forget any required equipment or chemical and if necessary sampling containers have to be prepared before.

Sampling containers should be chosen as listed below *(Table 1)* if they are used for one of the named parameters. All these preservation methods are according to EN ISO 5667-3 *(1995)* and might be extended if necessary (*e.g. for certain pharmaceuticals, organic compounds,...)*. Within the choice of container material it should be considered that some samples might develop gas and therefore glass material could propose a danger. Nevertheless all samples should be cooled as soon as possible, by store them already in some cooling boxes directly after the samples have been taken.

Parameter to determine	Material of sampling container P – Plastics, *e.g. polyethylene or PTFE* G – Glass *preferred brown-glass* BG – *Borsilicate*	Preservation method	Where to analyse?	Longest rec-ommended storage time	Comment	Internat. standard
Carbon, organic	G	acidification to pH<2 with H_2SO_4; cool to 2°C-5°C and store dark	Lab	1 week	The preservation method follows requirements of analytics. Determine immediately, if possible.	ISO 8245
	P	deep-freezing	Lab	1 month	in some cases it will be allowed to deep freeze (-20° C)	
Chloride	P or G	-	Lab	1 month	It might be necessary to determine before COD	ISO 9297

COD	P or G *(G preferred for low COD)*	acidification to pH<2 with H_2SO_4; cool to 2°C-5°C and store dark	Lab	5 days		ISO 6060
	P	freeze at -20°C	Lab	1 month		
Colour	P or G	-	Sampling Site	-		ISO 7887
Lipids, Oils, Hydrocarbons	G, *flushed with solvents (e.g. Pentane), cooled*	immediate extraction as possible, cool to 2°C-5°C	Lab	24h	It is recommended to add the solvent used for the extraction immediate after the sample has been collected or to carry out the extraction on the sampling site	
Kjeldahl-Nitrogen	P or BG	acidification to pH<2 with H_2SO_4; cool to 2°C-5°C and store dark	Lab	24 h	do not acidify if free ammonium shall be determined within the same sample	ISO 5663
Nitrate	P or G	acidification to pH<2; cool to 2° C-5°C	Lab	24 h		ISO 7890
Nitrite	P or G	cool to 2°C-5°C	Lab	24 h		ISO 6777
Oxygen	P or G	-	Sampling Site	-	ISO 5813; ISO 5814	
pH	P or G	-	Sampling Site		measurement should be immediately and if possible at sampling site	
		cool to 2°C-5°C	Lab	6 h		
Phosphorus, total	BG or G	cool to 2°C-5°C	Lab	24 h	for low concentrations use iodised glass containers (add some iodine-crystals and heat container to 60°C for 8h). *Iodine can be resolved! -> check up with analysts*	
		acidification to pH<2 with H_2SO_4	Lab	1 month	*see above*	
Settleable Solids	P or G	-	Lab	24 h	determination should be immediately and if possible at sampling site	
Total Solids	P or G	cool to 2°C-5°C	Lab	24 h		
Turbidity	P or G	-	Lab	24 h	determination should be if possible at sampling site	

Table 1: Preservation Methods according EN ISO 5667-3 (1995)

4. Sampling Protocol per Sewage Treatment Plant

It will be necessary to draw up a sampling protocol for each site to ensure that all samples taken on different days are comprehensive.

Former investigations already dealt with sampling from some of the selected WTP and therefore it might be useful taking 'fluid' samples from the same locations. Additional it is the task to take some biomass samples from different locations of each WTP. To receive a detailed outline of the occurrence and fate of pharmaceutical residues within these WTP's it is recommended taking samples from (nearly) each step where biomass is settled, *eg. primary, secondary, tertiary sludge.* Taking representative samples of sludge might pose problems, especially with big settlement tanks, because the 'sediment' should be well mixed before the sample is taken. If this isn't possible at all it has to be pointed up in the sampling protocol.

At this point of time it cannot yet be figured out where it will be the best location on each sampling site to receive representative biomass samples. This task will have to be discussed with labour of the sampling site. A so far general sampling protocol is given below and a list of interesting facts for each site is given in *chapter 2*. The protocol shall then be widen by local facts like for instance, *exact location of sampling (eg mark in flowdiagramm), depth of sampling.*

Sampling Protocol

Sewage Treatment Work	
Location of Sampling	

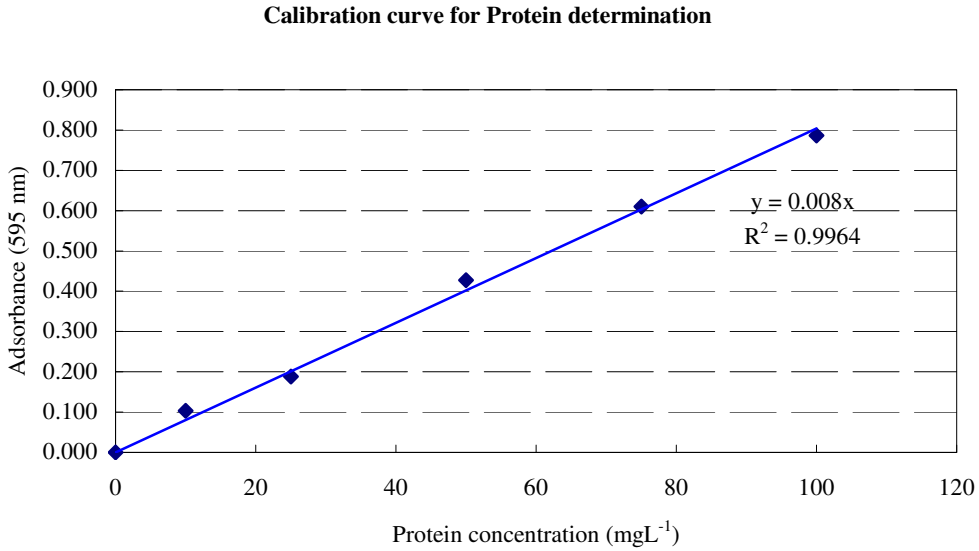
Date	
Hour	
Name of Sampler	

N° of sample	
Purpose of sample (parameters to determine)	
weather at time of sample	
outward appearance of sampling	
type of preservation	
storage condition of sample *(cool at 4°C, use before...)*	
comments	

*** Besides the actual weather at point of sampling the weather of two or three days before could also be mentioned in the protocol, if this could have an influence of the dilution of the sewage and therefor concentration of possible pharmaceutical residues.

Appendix 4.3.3.3 : Protein determination

Calibration (Peterson Method)



Concentration (mg/L)	Abs 1	Abs 2	Abs 3	Average	Abs st dev
0	0.000	0.000	0.000	0.000	0.000
10	0.102	0.105	0.103	0.103	0.002
25	0.262	0.261	0.041	0.188	0.127
50	0.426	0.429	0.428	0.428	0.002
75	0.620	0.610	0.600	0.610	0.010
100	0.787	0.788	0.785	0.787	0.002

Protein determination according Bradford (1976); Lowry et al. (1951)

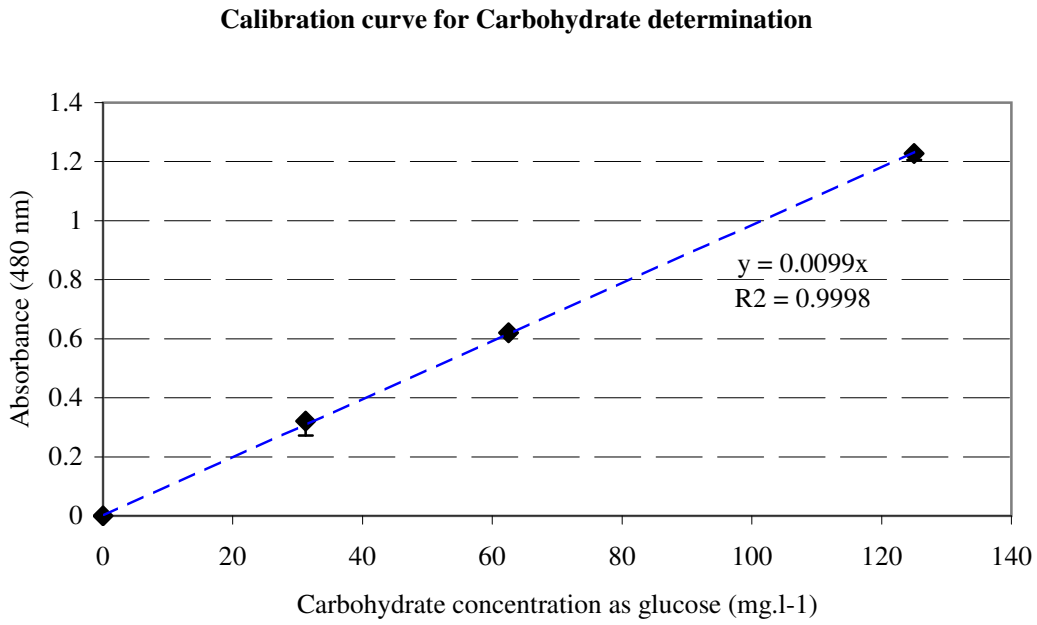
Bradford method

Protein concentrations by using the Bradford method (Bradford, 1976): 1 mL sample of suspended solid free supernatants was added to 1 mL of Bradford reagent (Sigma - Aldrich, Gillingham, UK). After 20 min -which are necessary to develop colour- the sample was diluted with 2 mL deionised water. The Adsorbance was measured against a blank at 595 nm in a Jenway 6505 UV/Visible Spectrophotometer and results were calculated from a calibration curve obtained from protein standard bovine serum albumin.

Lowry mehtod

Protein by using the Lowry-phenol method (Lowry et al., 1951): 0.2 mL of sample was mixed with 2.2 mL Biuret reagent (Sigma - Aldrich, Gillingham, UK) and kept for 10 min at room temperature. Then 0.1 mL of Folin and Ciocalteu's Phenol reagent was added and colour was allowed to developed for 30 min at room temperature. The samples were transferred to cuvetes and measured against a blank at 595 nm (Jenway 6505 UV/VIS). The concentration is calculated from a calibration curve obtained from protein standard.

Appendix 4.3.3.4 : Carbohydrate determination



Concentration (mg/L)	Abs 1	Abs 2	Abs 3	Average	Abs st dev
0	0.000	0.000	0.000	0.000	0.000
10	0.102	0.105	0.103	0.103	0.002
25	0.262	0.261	0.041	0.188	0.127
50	0.426	0.429	0.428	0.428	0.002
75	0.620	0.610	0.600	0.610	0.010
100	0.787	0.788	0.785	0.787	0.002

Appendix 5.2.1 - Full Scale Sampling List - Site A

Sampling point: ***Influent - A1***

DATE Time	25-Mar-04	30-Apr-04 14:20	21-May-04 15:00	11-Jun-04 12:50	01-Jul-04 13:08	15-Jul-04 15:13
Site Conditions						
Temperature	10.1	-	14.10	15.60	16.00	17.10
pH	8.35	-	8.20	8.15	8.25	8.08
COD						
COD [mgL^{-1}]	**140.0**	**54.0**	**250.0**	**223.0**	**244.0**	**130.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	**1.7**	-	**1.8**	**0.05**
std [+/-]	-	-	-	-	-	0.0
Nitrite						
NO_2-N [mgL^{-1}]	-	-	**0.08**	-	-	**1.19**
std [+/-]	-	-		-	-	0.2
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**14.4**	**6.8**	**51.2**	**16.9**	**10.1**	**5.8**
std [+/-]	**1.6**	**1.1**	**1.5**	**2.4**	**0.1**	**1.1**
Soluble Proteins						
Soluble Proteins [mgL^{-1}]	**7.5**	**11.8**	**28.6**	**34.8**	**27.1**	**29.4**
std [+/-]	**7.8**	**0.4**	**1.4**	**2.7**	**1.1**	**0.4**
Carbon						
TOC [ppm]	103.9					
TC [ppm]	104.5					
IC [ppm]	0.6					
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	1282	594	1948	1371	4945	897

Sampling point: ***RBC Influent - A2***

DATE Time	25-Mar-04	30-Apr-04 14:45	21-May-04 15:05	11-Jun-04 13:08	01-Jul-04 13:15	15-Jul-04 15:25
Site Conditions	No Sample due to oily surface					
Temperature		-	16.3	17.0	16.9	17.6
pH		-	7.39	7.06	7.10	7.16
COD						
COD [mgL^{-1}]		**33.0**	**270.0**	**205.0**	**114.0**	**112.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	**1.8**	-	**1.6**	**0.12**
std [+/-]	-	-		-	-	**0.0**
Nitrite						
NO_2-N [mgL^{-1}]	-	-	**0.41**	-	-	**1.16**
std [+/-]	-	-		-	-	**1.5**
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]		**1.97**	**16.46**	**13.48**	**9.29**	**5.56**
std [+/-]		**0.1**	**2.1**	**2.5**	**0.9**	**0.4**
Soluble proteins						
Soluble Proteins [mgL^{-1}]		**8.1**	**38.8**	**31.3**	**21.2**	**21.7**
std [+/-]		**0.1**	**0.1**	**3.2**	**0.4**	**0.2**
Carbon						
TOC [ppm]						
TC [ppm]						
IC [ppm]						
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**no data**	**526**	**136**	**1455**	**1865**	**1939**

Sampling point: ***RBC Effluent - A3***

DATE	25-Mar-04	30-Apr-04	21-May-04	11-Jun-04	01-Jul-04	15-Jul-04
Time		14:35	14:55	13:00	13:11	15:20
Site Conditions						
Temperature	10.2	-	15.4	18.1	18.1	18.4
pH	7.50	-	7.2	7.0	7.0	7.3
COD						
COD [mgL^{-1}]	**31.0**	**26.0**	**78.0**	**35.0**	**32.0**	**37.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	**31.8**	-	**22.4**	**94.00**
std [+/-]	-	-	-	-	-	**0.1**
Nitrite						
NO_2-N [mgL^{-1}]	-	-	**0.36**	-	-	**17.48**
std [+/-]	-	-	-	-	-	**0.6**
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**13.33**	**4.55**	**9.75**	**14.04**	**7.53**	**6.31**
std [+/-]	**1.1**	**0.1**	**2.8**	**12.9**	**0.4**	**1.2**
Soluble proteins						
Soluble Proteins [mgL^{-1}]	**6.4**	**10.1**	**15.0**	**8.8**	**7.0**	**10.7**
std [+/-]	**2.4**	**0.0**	**0.3**	**0.2**	**0.6**	**0.5**
Carbon						
TOC [ppm]	9.7					
TC [ppm]	53.2					
IC [ppm]	43.4					
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**310**	**106**	**256**	**272**	**66**	**52**

Sampling point: ***Final Effluent - A4***

DATE	25-Mar-04	30-Apr-04	21-May-04	11-Jun-04	01-Jul-04	15-Jul-04
Time		14:15	14:45	12:38	13:03	15:05
Site Conditions						
Temperature	9.3	-	15.0	15.0	15.0	16.3
pH	7.67	-	7.25	7.41	7.41	7.33
COD						
COD [mgL^{-1}]	**17.0**	**19.0**	**78.0**	**22.0**	**24.0**	**29.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	**5.3**	-	**10.6**	**39.89**
std [+/-]	-	-		-	-	**1.5**
Nitrite						
NO_2-N [mgL^{-1}]	-	-	**0.10**	-	-	**13.71**
std [+/-]	-	-		-	-	**6.0**
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**7.37**	**1.21**	**6.92**	**0.40**	**1.67**	**1.36**
std [+/-]	**0.6**	**0.3**	**0.2**	**0.3**	**0.1**	**0.1**
Soluble Proteins						
Soluble Proteins [mgL^{-1}]	**7.0**	**7.7**	**10.8**	**6.1**	**3.5**	**9.5**
std [+/-]	**0.3**	**0.3**	**0.8**	**0.1**	**0.3**	**0.2**
Carbon						
TOC [ppm]	49.1					
TC [ppm]	49.9					
IC [ppm]	0.8					
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**267**	**194**	**289**	**322**	**75**	**n.d.**

Sampling point: ***RBC sludge - A5***

DATE Time	25-Mar-04	30-Apr-04 14:30	21-May-04 15:05	11-Jun-04 13:18	01-Jul-04	15-Jul-04 15:30
Site Conditions **COD**						
COD_{SMP} [mgL^{-1}]	**1156.0**	**2616.0**	**3605.0**	**2450.0**	**1852.0**	**2290.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	-	-	**4.2**	**0.72**
std [+/-]	-	-	-	-	-	**0.2**
Nitrite						
NO_2-N [mgL^{-1}]	-	-	-	-	-	**3.80**
std [+/-]	-	-	-	-	-	**0.0**
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**106.4**	**147.7**	**125.5**	**147.7**	**122.6**	**120.4**
std [+/-]	**11.6**	**5.4**	**3.6**	**43.9**	**1.1**	**2.3**
Soluble Proteins						
Soluble Proteins [mgL^{-1}]	**228.5**	**428.1**	**268.6**	**417.3**	**321.4**	**423.0**
standard deviation [mgL^{-1}]	**29.0**	**1.4**	**3.9**	**1.7**	**4.7**	**8.0**
EPS - Extraction			*micro-centrifuge*			
weight sludge [g]	10.0	broken	0.20	0.20	3	3
volume sludge [ml]		centrifuge	1.5	1.5		
volume DI water [ml]	50.0		1.0	1.0	200	200.0
EPS - COD						
dilution 1:x		1	1	1	3	4
measured		820	5725	3090	480	382.0
COD_{EPS} [mgL^{-1}]		**820**	**5725**	**3090**	**1440**	**1528.0**
Extracellular Carbohydrates						
mg Carbohydrates/g TS	**21.0**		**38.3**	**32.3**	**35.3**	**27.6**
std [+/-]	1.0		0.8	1.4	1.3	0.1
mg Carbohydrates/g VTS	**25.1**		**47.5**	**38.5**	**41.2**	**32.9**
std [+/-]	1.1		0.9	1.6	1.5	0.1
Extracellular Proteins						
mg Protein/g TS	**67.0**		**55.8**	**43.8**	**68.6**	**69.2**
std [+/-]	**0.6**		**0.6**	**0.5**	**2.0**	**0.3**
mg Protein/g VTS	**80.2**		**69.2**	**52.1**	**80.0**	**82.4**
std [+/-]	**0.7**		**0.8**	**0.6**	**2.3**	**0.3**
Ratio EPS_P/EPS_C						
	3.2		**1.5**	**1.4**	**1.9**	**2.5**
Total&Volatile Solids per mg/g						
TS [g/kg]	**69**	not determined	**70**	**60**	**88**	**91**
VTS [%]	**83.6**		**80.7**	**84.0**	**85.7**	**83.9**

Calculation: ***Triclosan load per day and per capita***

Site A (PE: 405; average flow: 120 m^3d^{-1})							
date	Triclosan concentration ngL^{-1}		load of Triclosan g/day		Triclosan µg/day per capita		Overall removall rate [%]
	Influent	Final Eflluent	Influent	Final Eflluent	Influent	Final Eflluent	
	Tric A1	Tric A4	Tric A1	Tric A4	Tric A1	Tric A4	
25-Mar-04	1282	267	0.2	0.032	380	79	*79.2*
30-Apr-04	594	194	0.1	0.023	176	57	*67.3*
21-May-04	1948	289	0.2	0.035	577	86	*85.2*
11-Jun-04	1371	322	0.2	0.039	406	95	*76.5*
01-Jul-04	4945	75	0.6	0.009	1465	22	*98.5*
15-Jul-04	897		0.1	0.000	266		~~*100.0*~~
average	*1840*	*229*	*0.22*	*0.0229*	*545*	*68*	*81.3*

date	**Triclosan concentration ngL-1**			
	Influent	RBC Influent	RBC Effluent	Final Eflluent
	Tric A1	Tric A2	Tric A3	Tric A4
25-Mar-04	1282		310.0	267
30-Apr-04	594	526.0	106.0	194
21-May-04	1948	136.0	256.0	289
11-Jun-04	1371	1455.0		322
01-Jul-04	4945	1865.0	66.0	75
15-Jul-04	897	1939.0	52.0	
average	*1840*	*1184*	*158*	*229*

date	**load of Triclosan g/day**			
	Influent	RBC Influent	RBC Effluent	Final Eflluent
	Tric A1	Tric A2	Tric A3	Tric A4
25-Mar-04	0.154		0.037	0.032
30-Apr-04	0.071	0.063	0.013	~~0.023~~
21-May-04	0.234	0.016	0.031	~~0.035~~
11-Jun-04	0.165	0.175		~~0.039~~
01-Jul-04	0.593	0.224	0.008	0.009
15-Jul-04	0.108	0.233	0.006	0.000
average	*0.221*	*0.142*	*0.019*	*0.016*

Appendix 5.2.2 - Full Scale Sampling List - Site C

Sampling point: ***Influent - C1***

Time	25-Mar-04	30-Apr-04 11:30	21-May-04 12:40	11-Jun-04 10:41	01-Jul-04 11:00	15-Jul-04 13:10
Site Conditions						
Temperature	9.5	-	12.7	15.6	15.9	16.2
pH	8.21	-	7.64	7.80	7.91	7.61
COD						
COD [mgL^{-1}]	**56.0**	**41.0**	**274.0**	**134.0**	**77.0**	**295.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	**1.7**	-	**1.6**	**0.01**
std [+/-]	-	-	-	-	-	0.0
Nitrite						
NO_2-N [mgL^{-1}]	-	-	**0.05**	-	-	**3.8**
std [+/-]	-	-	-	-	-	2.8
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**19.3**	**6.7**	**8.9**	**6.5**	**4.6**	**9.9**
std [+/-]	5.8	0.2	1.9	2.0	1.1	1.1
Soluble Proteins						
Soluble Proteins [mgL^{-1}]	**23.8**	**12.9**	**40.5**	**27.0**	**17.8**	**44.8**
std [+/-]	21.5	0.3	0.2	0.9	1.0	1.0
Carbon						
TOC [ppm]	112.3					
TC [ppm]	114.3					
IC [ppm]	2.026					
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**2100**	**1562**	**n.d.**	**3057**	**1703**	**2202**

Sampling point: ***Settler - C2***

DATE Time	25-Mar-04	30-Apr-04 11:40	21-May-04 12:45	11-Jun-04 10:49	01-Jul-04 11:10	15-Jul-04 13:20
Site Conditions						
Temperature	9.0	-	14.5	15.5	16.1	16.7
pH	7.67	-	7.50	7.90	7.39	7.65
COD						
COD [mgL^{-1}]	**74.0**	**24.0**	**94.0**	**102.0**	**168.0**	**104.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	**18.3**	-	**2.8**	**0.03**
std [+/-]	-	-	-	-	-	0.0
Nitrite						
NO_2-N [mgL^{-1}]	-	-	**0.7**	-	-	**6.8**
std [+/-]	-	-	-	-	-	0.1
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**22.78**	**6.87**	**11.92**	**7.68**	**9.55**	**5.96**
std [+/-]	3.2	1.7	0.6	1.3	0.4	0.1
Soluble proteins						
Soluble Proteins [mgL^{-1}]	**7.6**	**11.73**	**17.8**	**24.8**	**27.8**	**26.9**
std [+/-]	1.5	0.9	1.0	0.5	0.0	3.1
Carbon						
TOC [ppm]	~~92.280~~					
TC [ppm]	~~92.110~~					
IC [ppm]	~~-0.167~~					
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**1141**	**1118**	**40**	**1779**	**141**	**1773**

Sampling point: ***Humus Effluent - C3***

DATE Time	25-Mar-04	30-Apr-04 11:48	21-May-04 13:05	11-Jun-04 10:55	01-Jul-04 11:20	15-Jul-04 13:20
Site Conditions						
Temperature	8.1	-	14.5	17.5	16.5	17.2
pH	7.50	-	7.50	7.50	7.15	7.27
COD						
COD [mgL^{-1}]	**40.0**	**10.0**	**94.0**	**39.0**	**42.0**	**48.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	**34.2**	-	**35.1**	**144.1**
std [+/-]	-	-	-	-	-	**2.6**
Nitrite						
NO_2-N [mgL^{-1}]	-	-	**0.28**	-	-	**20.0**
std [+/-]	-	-	-	-	-	**3.6**
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**21.16**	**5.15**	**8.03**	**7.93**	**8.89**	**7.88**
std [+/-]	**2.8**	**0.3**	**1.1**	**1.5**	**0.4**	**0.0**
Soluble proteins						
Soluble Proteins [mgL^{-1}]	**11.9**	**8.83**	**16.0**	**9.8**	**7.7**	**11.3**
std [+/-]	**2.9**	**0.3**	**0.1**	**0.2**	**0.9**	**0.6**
Carbon						
TOC [ppm]	59.750					
TC [ppm]	60.320					
IC [ppm]	0.566					
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**283**	**186**	**244**	**22**	**141**	**116**

Sampling point: ***Final Effluent - C4***

DATE Time	25-Mar-04	30-Apr-04 11:55	21-May-04 14:45	11-Jun-04 11:00	01-Jul-04 11:30	15-Jul-04 13:35
Site Conditions						
Temperature	8.4	-	15.0	16.9	16.8	16.2
pH	7.73	-	7.25	7.42	7.38	7.25
COD						
COD [mgL^{-1}]	**33.0**	**29.0**	**90.0**	**34.0**	**37.0**	**47.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	**33.1**	-	**32.8**	**135.0**
std [+/-]	-	-	-	-	-	1.0
Nitrite						
NO_2-N [mgL^{-1}]	-	-	0.34	-	-	6.9
std [+/-]	-	-	-	-	-	**2.2**
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**24.14**	**6.72**	**8.38**	**8.79**	**8.33**	**7.53**
std [+/-]	**2.0**	**1.9**	**1.1**	**2.1**	**0.1**	**0.8**
Soluble Proteins						
Soluble Proteins [mgL^{-1}]	**21.1**	**8.8**	**13.9**	**10.2**	**8.7**	**11.5**
std [+/-]	**12.6**	**0.9**	**0.1**	**0.7**	**1.7**	**1.3**
Carbon						
TOC [ppm]	57.60					
TC [ppm]	59.02					
IC [ppm]	1.41					
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**290**	**254**	**191**	**35**	**117**	**42**

Calculation: ***Triclosan load per day and per capita***

Site C PE: 2,307; average flow: 1,002 m^3d^{-1}							
date	Triclosan concentration ngL^{-1}		load of Triclosan g/day		Triclosan µg/day per capita		Overall removall rate [%]
	Influent	Final Effluent	Influent	Final Effluent	Influent	Final Effluent	
	Tric C1	Tric C4	Tric C1	Tric C4	Tric C1	Tric C4	
25-Mar-04	2100	290.0	2.1	0.291	912	126	*86.2*
30-Apr-04	1562	254.0	1.6	0.255	678	110	*83.7*
21-May-04		191.0		0.191		83	
11-Jun-04	3057	35.0	3.1	0.035	1328	15	*98.9*
01-Jul-04	1703	117.0	1.7	0.117	740	51	*93.1*
15-Jul-04	2202	42.0	2.2	0.042	956	18	*98.1*
average	*2125*	*155*	*2.1*	*0.16*	*923*	*67*	*92*

date	Triclosan concentration ngL-1			
	Influent	Settler	Humus Effluent	Final Effluent
	Tric C1	Tric C2	Tric C3	Tric C4
25-Mar-04	2100	1141	283	290
30-Apr-04	1562	1118	186	254
21-May-04		40	244	191
11-Jun-04	3057	1779	22	35
01-Jul-04	1703	141	141	117
15-Jul-04	2202	1773	116	42
average	*2125*	*999*	*165*	*155*

date	load of Triclosan g/day			
	Influent	Settler	Humus Effluent	Final Effluent
	Tric C1	Tric C2	Tric C3	Tric C4
25-Mar-04	2.104	1.143	0.283	~~0.291~~
30-Apr-04	1.565	1.120	0.186	~~0.255~~
21-May-04		0.040	0.244	0.191
11-Jun-04	3.063	1.783	0.022	0.035
01-Jul-04	1.706	0.141	0.141	0.117
15-Jul-04	2.206	1.777	0.116	0.042
average	*2.129*	*1.001*	*0.166*	*0.096*

Appendix 5.2.3 - Full Scale Sampling List - Site D

Sampling point: ***Influent 1 - D1***

DATE Time	25-Mar-04	30-Apr-04 9:45	21-May-04	11-Jun-04 9:33	01-Jul-04	15-Jul-04	30-Jul-04 10:30
Site Conditions	**No Sampling**		**No Sampling**			**No Sampling**	
Temperature		-		16.3	17.5		-
pH		-		7.68	7.57		7.45
COD							
COD [mgL^{-1}]		**86.0**		**151.0**	**302.0**		**142.0**
Nitrate							
NO_3-N [mgL^{-1}]		**-**		**-**	**1.8**		**1.0**
std [+/-]		-		-	-		1.2
Nitrite							
NO_2-N [mgL^{-1}]		**-**		**-**	**-**		**20.6**
std [+/-]		-		-	-		9.5
Soluble Carbohydrates							
Carbohydrates [mgL^{-1}]		**28.28**		**51.62**	**36.21**		**10.10**
std [+/-]		1.4		0.3	0.6		0.7
Soluble Proteins							
Proteins [mgL^{-1}]		**24.01**		**50.06**	**41.91**		**24.01**
std [+/-]		0.3		0.1	3.1		0.8
Ratio Soluble Proteins/Carbohydrates							
		0.8		1.0	1.2		2.4
Suspended & Volatile SSolids							
SS [mgL^{-1}]		**-**	**-**	**-**	**-**		**201.2**
VSS [%]		**-**	**-**	**-**	**-**		**93.4**
Carbon							
TOC [ppm]		-	-	-	-		-
TC [ppm]		-	-	-	-		-
IC [ppm]		-	-	-	-		-
Traced Pharmaceutical							
Triclosan [ngL-1]		**728**		**164**	**2504**		**1575**

Sampling point: ***Influent 2 - D2***

DATE Time	25-Mar-04	30-Apr-04 10:30	21-May-04	11-Jun-04 9:43	01-Jul-04 10:00	15-Jul-04	30-Jul-04 10:30
Site Conditions	**No Sampling**		**No Sampling**			**No Sampling**	
Temperature		-		12.4	18.0		-
pH		-		7.13	8.00		6.83
COD							
COD [mgL^{-1}]		**56.0**		**141.0**	**148.0**		**222.0**
Nitrate							
NO_3-N [mgL^{-1}]		**-**		**-**	**1.7**		**1.7**
std [+/-]		-		-	-		2.4
Nitrite							
NO_2-N [mgL^{-1}]		**-**		**-**	**-**		**27.7**
std [+/-]		-		-	-		6.6
Soluble Carbohydrates							
Carbohydrates [mgL^{-1}]		**15.71**		**24.49**	**15.30**		**30.71**
std [+/-]		3.5		1.6	0.6		0.4
Soluble Proteins							
Proteins [mgL^{-1}]		**16.05**		**37.16**	**28.46**		**44.51**
std [+/-]		2.4		0.0	0.1		0.6
Ratio Soluble Proteins/Carbohydrates							
		1.0		1.5	1.9		1.4
Suspended & Volatile Solids							
SS [mgL^{-1}]							**193.0**
VSS [%]							**94.8**
Carbon							
TOC [ppm]							
TC [ppm]							
IC [ppm]							
Traced Pharmaceutical							
Triclosan [ngL-1]		**386**		**71**	**1729**		**2219**

Sampling point: S***ettler - D3***

DATE Time	25-Mar-04	30-Apr-04	21-May-04	11-Jun-04 9:15	01-Jul-04	15-Jul-04	30-Jul-04 10:20
Site Conditions	**No Sampling**		**No Sampling**			**No Sampling**	
Temperature		-		17.2	17.8		-
pH		-		7.32	7.63		6.95
COD							
COD [mgL^{-1}]		**59.0**		**180.0**	**120.0**		**152.0**
Nitrate							
NO_3-N [mgL^{-1}]	-	**-**		**-**	**1.6**		**2.8**
std [+/-]		-		-	-		**3.9**
Nitrite							
NO_2-N [mgL^{-1}]	-	**-**		**-**	**-**		**9.5**
std [+/-]		-		-	-		**2.6**
Soluble Carbohydrates							
Carbohydrates [mgL^{-1}]		**13.79**		**20.66**	**13.18**		**11.77**
std [+/-]		**5.1**		**0.8**	**0.1**		**5.1**
Soluble Proteins							
Proteins [mgL^{-1}]		**14.63**		**39.94**	**25.00**		**27.16**
std [+/-]		**0.4**		**1.8**	**1.8**		**1.4**
Ratio Soluble Proteins/Carbohydrates							
		1.1		**1.9**	**1.9**		**2.3**
Suspended & Volatile SSolids							
SS [mgL^{-1}]							**112.7**
VSS [%]							**91.6**
Carbon							
TOC [ppm]							
TC [ppm]							
IC [ppm]							
Traced Pharmaceutical							
Triclosan [ngL-1]		**187**		**73**	**930**		**776**

Sampling point: Final Effluent ***- D7***

DATE Time	25-Mar-04	30-Apr-04 10:15	21-May-04	11-Jun-04 9:18	01-Jul-04	15-Jul-04	30-Jul-04 10:25
Site Conditions	**No Sampling**		**No Sampling**			**No Sampling**	
Temperature		-		18.0	17.7	-	
pH		-		6.45	6.45		6.5
COD							
COD [mgL^{-1}]		**11.0**		**25.0**	**26.0**		**80.0**
Nitrate							
NO_3-N [mgL^{-1}]	-	**-**		**-**	**20.3**		**n.d.**
std [+/-]	-	-		-	-		
Nitrite							
NO_2-N [mgL^{-1}]	-	**-**		**-**	**-**		**14.6**
std [+/-]	-	-		-	-		**0.8**
Soluble Carbohydrates							
Carbohydrates [mgL^{-1}]		**3.79**		**6.52**	**3.74**		**3.23**
std [+/-]		**0.9**		**4.2**	**0.0**		**0.4**
Soluble proteins							
Proteins [mgL^{-1}]		**5.74**		**11.23**	**0.74**		**7.72**
std [+/-]		0.1		0.9	0.5		0.1
Ratio Soluble Proteins/Carbohydrates							
		1.5		**1.7**	**0.2**		**2.4**
Suspended & Volatile SSolids							
SS (mgL^{-1})							**13.3**
VSS (%)							**51.2**
Carbon							
TOC [ppm]							
TC [ppm]							
IC [ppm]							
Traced Pharmaceutical							
Triclosan [ngL-1]		**61**		**61**	**371**		**59**

Sampling point: ***ANOX - D4***

DATE Time	25-Mar-04	30-Apr-04	21-May-04	11-Jun-04 9:22	01-Jul-04	15-Jul-04	30-Jul-04 10:08
Site Conditions		not determined	**No Sampling**			**No Sampling**	
Temperature	12.5			17.5	18.3		-
pH	7.10			6.90	7.10		7.45
COD							
dilution	1			1	1		1
measured	49.0			154.0	78.0		116.0
COD [mgL^{-1}]	**49.0**			**154.0**	**78.0**		**116.0**
Nitrate							
NO_3-N [mgL^{-1}]	-	-		-	**12.1**		**6.0**
std [+/-]	-	-		-	-		**1.9**
Nitrite							
NO_2-N [mgL^{-1}]	-	-		-	-		**14.8**
std [+/-]	-	-		-	-		**8.3**
Soluble Carbohydrates							
Carbohydrates [mgL^{-1}]	**18.79**			**30.61**	**6.16**		**6.16**
std [+/-]	**9.4**			-	**1.4**		**0.0**
Soluble Proteins							
Proteins [mgL^{-1}]	**19.4**			**4.94**	**7.78**		**17.16**
std [+/-]	**1.0**			**0.2**	**0.9**		**1.2**
Ratio Soluble Proteins/Carbohydrates							
	1.0			**0.2**	**1.3**		**2.8**
EPS - Extraction							
comments				microcentrifuged			
EPS - COD							
COD_{EPS} [mgL^{-1}]	-			**380.0**	**355.0**		**476.0**
Extracellular Carbohydrates							
mg EPS-Carbohydrates/g SS	-			**41.8**	**11.8**		**14.7**
std [+/-]	-			*3.5*	*0.2*		*0.3*
mg EPS-Carbohydrates/g VSS	-			**60.7**	**17.2**		**22.5**
std [+/-]	-			*10.0*	*0.5*		*0.9*
Extracellular Proteins							
mg EPS-Proteins/g SS	-			**40.4**	**21.6**		**34.3**
std [+/-]	-			*5.3*	*0.1*		*0.9*
mg EPS-Proteins/g VSS	-			**58.8**	**31.5**		**52.6**
std [+/-]	-			*7.7*	*0.1*		*1.4*
Ratio EPS_P/EPS_C							
	-			0.97	1.83		2.34
Total&Volatile Solids per mg/g							
TS [mg/g wet sludge]					**75.5**		**75.0**
VTS [%]					**68.6**		**66.5**
Total&Volatile Solids per mg/L							
TS [mgL^{-1}]				**3.9**	**3.8**		-
VTS [%]				**61.9**	**64.4**		-
Suspended & Volatile SSolids							
SS [mgL^{-1}]				**2792.9**	**2910.6**		**3381.7**
VSS [%]				**68.8**	**68.5**		**65.2**
Carbon							
TOC [ppm]	13.6						
TC [ppm]	82.9						
IC [ppm]	69.4						
Particle Size							
specific surface [m^2/g]	0.057			0.0547	0.0688		0.0676
d (0.1)	64.622			66.065	54.023		60.711
d (0.9)	523.031			476.138	399.674		337.271
average diameter d (0.5)	**228.557**			**204.144**	**152.049**		**149.857**
Traced Pharmaceutical							
Triclosan [ngL-1]	-			**110**	**127.0**		**94.0**

Sampling point: ***AEROB - D5***

DATE Time	25-Mar-04	30-Apr-04 10:05	21-May-04	11-Jun-04 9:25	01-Jul-04	15-Jul-04	30-Jul-04 10:10
Site Conditions	**No Sampling**		**No Sampling**			**NO Sampling**	
Temperature		-		18.4	18.5		-
pH		-		6.40	6.27		7.25
COD							
COD [mgL^{-1}]		**21.0**		**94.0**	**86.0**		**82.0**
Nitrate							
NO_3-N [mgL^{-1}]	-	**-**		**-**	**2.6**		**35.9**
std [+/-]	-	-		-	-		**0.6**
Nitrite							
NO_2-N [mgL^{-1}]	-	**-**		**-**	**-**		**13.2**
std [+/-]	-	-		-	-		**2.2**
Soluble Carbohydrates							
Carbohydrates [mgL^{-1}]		**3.28**		**6.36**	**7.07**		**3.59**
std [+/-]		**0.8**		**1.9**	**0.3**		**0.4**
Soluble Proteins							
Proteins [mgL^{-1}]		**6.98**		**11.11**	**12.96**		**10.49**
std [+/-]		**0.3**		**0.0**	**0.3**		**0.2**
Ratio Soluble Proteins/Carbohydrates							
		2.1		**1.7**	**1.8**		**2.9**
EPS - Extraction							
comments		broken centrifuge		**micro centrifuged**			
EPS - COD							
COD_{EPS} [mgL^{-1}]				**475.00**	**765.00**		**482.00**
Extracellular Carbohydrates							
mg EPS-Carbohydrates/g SS				**39.2**	**5.6**		**12.9**
std [+/-]				*1.8*	*0.1*		*0.1*
mg EPS-Carbohydrates/g VSS				**57.4**	**7.9**		**13.2**
std [+/-]				*5.4*	*0.4*		*0.2*
Extracellular Proteins							
mg EPS-Proteins/g SS				**37.7**	**13.5**		**42.4**
std [+/-]				*0.3*	*2.9*		*0.1*
mg EPS-Proteins/g VSS				**55.1**	**19.1**		**43.3**
std [+/-]				*0.4*	*4.1*		*0.1*
Ratio EPSp/EPSc							
				0.96	2.41		3.28
Total&Volatile Solids per mg/g							
TS [mg/g wet sludge]					**77.9**		**73.8**
VTS [%]					**72.1**		**66.0**
Total&Volatile Solids per mg/L							
TS [mgL^{-1}]				**4.0**	**3.9**		
VTS [%]				**61.2**	**66.8**		
Suspended & Volatile Solids							
SS [mgL^{-1}]				**2898.4**	**3357.8**		**3593.3**
VSS [%]				**68.4**	**70.6**		**98.0**
Carbon							
TOC [ppm]							
TC [ppm]							
IC [ppm]							
Particle Size							
specific surface [m^2/g]				0.0526	0.0646		0.0478
d (0.1)				72.55	58.767		77.546
d (0.9)				492.849	418.337		565.084
average diameter d (0.5)				**209.728**	**169.165**		**224.399**
Traced Pharmaceutical							
Triclosan [ngL-1]		**83.0**		**12.0**	**40.0**		**67.0**

Sampling point: ***RAS - D6***

DATE Time	25-Mar-04	30-Apr-04	21-May-04	11-Jun-04 9:22	01-Jul-04	15-Jul-04	30-Jul-04 10:16
Site Conditions	**No Sampling**	not determined	**No Sampling**			**NO Sampling**	
Temperature				17.5	18.5		-
pH				6.90	6.80		7.33
COD							
COD [mgL^{-1}]				154.0	102.0		116.0
Nitrate							
NO_3-N [mgL^{-1}]				-	7.9		14.3
std [+/-]				-	-		2.4
Nitrite							
NO_2-N [mgL^{-1}]				-	-		13.2
std [+/-]				-	-		8.2
Soluble Carbohydrates							
Carbohydrates [mgL^{-1}]				**8.38**	**6.06**		**6.67**
std [+/-]				**0.1**	**0.6**		**1.1**
Soluble Proteins							
Proteins [mgL^{-1}]				**5.99**	**10.62**		**15.86**
std [+/-]				**0.1**	**0.9**		**0.1**
EPS - Extraction							
comments				broken centrifuge			
EPS - COD							
COD_{EPS} [mgL^{-1}]				805.00	690.0		854.00
Extracellular Carbohydrates							
std [+/-]				49.3	0.9		6.6
mg EPS-Carbohydrates/g SS				**35.6**	**7.4**		**19.8**
std [+/-]				*4.5*	*0.1*		*0.8*
mg EPS-Carbohydrates/g VSS				**52.2**	**10.5**		**28.5**
std [+/-]				*13.1*	*0.2*		*2.3*
Extracellular Proteins							
mg EPS-Proteins/g SS				**18.8**	**14.5**		**44.4**
std [+/-]				*0.9*	*1.8*		*0.2*
mg EPS-Proteins/g VSS				**27.6**	**20.8**		**63.9**
std [+/-]				*1.4*	*2.5*		*0.3*
Ratio EPSp/EPSc							
				0.53	1.97		2.24
Total&Volatile Solids per mg/g							
TS [mg/g wet sludge]					**89.0**		**84.1**
VTS [%]					**69.4**		**65.6**
Total&Volatile Solids per mg/L							
TS [mgL^{-1}]				**6.6**	**6.2**		
VTS [%]				**64.3**	**66.3**		
Suspended & Volatile Solids							
SS [mgL^{-1}]				**5518**	**6055**		**4210.0**
VSS [%]				**68.2**	**69.8**		**69.5**
Particle Size							
specific surface [m^2/g]				0.0449	0.0727		0.0571
d (0.1)				81.796	49.967		66.354
d (0.9)				640.649	388.67		450.76
average diameter d (0.5)				**260.754**	**148.937**		**179.876**
Carbon							
TOC [ppm]							
TC [ppm]							
IC [ppm]							
Traced Pharmaceutical							
Triclosan [ngL-1]				**n.d.**	**n.d.**		**59**

Calculation: ***Triclosan load per day and per capita***

Site D PE: 494,387; average flow: 125,000 m^3d^{-1}							
date	Triclosan concentration ngL^{-1}		load of Triclosan g/day		Triclosan µg/day per capita		Overall removall rate [%]
	Influent	Final Effluent	Influent	Final Effluent	Influent	Final Effluent	
	MW (D1+D2)	Tric D6	MW (D1+D2)	Tric D6	MW (D1+D2)	Tric D6	
25-Mar-04							
30-Apr-04	557	61.0	69.6	7.625	141	15	*89.0*
21-May-04							
11-Jun-04	118	61.0	14.7	7.625	30	15	*48.1*
01-Jul-04	2117	371.0	264.6	46.375	535	94	*82.5*
30-Jul-04	*1897*	*59*	237.1	7.375	480	15	*96.9*
average	*1172*	*138*	*147*	*17.3*	*296*	*35*	*79*
adjusted effl. average *	*1172*	*77*	*147*	*7.5*	*296*	*15*	*93.45*

*adjusted eff average: necleting effluent values higher than anox-sample

Triclosan concentration ngL-1						
Sowe Inlet	Shareborne Inlet	Settler	ML ANOX	ML AEROB	Final Effluent	RAS
Tric D1	Tric D2	Tric D3	Tric D4	Tric D5	Tric D6	Tric D7
728	386	187		83	61	
164.00	71.0	73.0	110.0	12.0	61.0	n.d.
2504.00	1729.0	930.0	127.0	40.0	371.0	n.d.
1575.00	2219.0	776.0	94.0	67.0	59.0	59.0
1243	*1101*	*492*	*110*	*51*	*138*	*59*

load of Triclosan g/day							
Sowe Inlet	Shareborne Inlet	D1+D2	Settler	ML ANOX	ML AEROB	Final Effluent	RAS
Tric D1	Tric D2	(1:1)	Tric D3	Tric D4	Tric D5	Tric D6	Tric D7
91.000	48.250	69.625	23.375		10.375	7.625	
20.500	8.875	14.688	9.125	13.750	~~1.500~~	7.625	
313.000	216.125	264.563	116.250	15.875	5.000	~~46.375~~	
196.875	277.375	237.125	97.000	11.750	8.375	7.375	~~7.375~~
155.34	*137.66*	*146.50*	*61.44*	*13.79*	*7.92*	*7.54*	~~*7.38*~~

Appendix 5.2.4-1 - Full Scale Sampling List - Site B - grab samples

Sampling point: ***Influent 1 - B1***

DATE Time	25-Mar-04	30-Apr-04 12:45	21-May-04 13:50	11-Jun-04 11:32	01-Jul-04 12:30	15-Jul-04 13:58
Site Conditions						
Temperature	10.7	-	15.2	16.8	17.3	20.2
pH	8.95	-	7.85	8.47	8.10	7.94
COD						
COD [mgL^{-1}]	**200.0**	**114.0**	**384.0**	**195.0**	**302.0**	**240.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-		-	**2.3**	**0.6**
std [+/-]	-	-		-	-	**0.6**
Nitrite						
NO_2-N [mgL^{-1}]	-	-		-	-	**11.9**
std [+/-]	-	-		-	-	**9.0**
Soluble Carbohydrates						
Carbohydrates [mgL^{-1}]	**14.44**	**4.70**	**13.84**	**8.54**	**5.96**	**6.82**
std [+/-]	**0.4**	**0.4**	**1.1**	**1.4**	**0.3**	**0.1**
Soluble Proteins						
Proteins [mgL^{-1}]	**83.5**	**24.0**	**49.0**	**44.9**	**40.1**	**42.2**
std [+/-]	**7.8**	**0.3**	**1.7**	**0.3**	**0.2**	**0.9**
Ratio Soluble Proteins/Carbohydrates						
	5.8	**5.1**	**3.5**	**5.3**	**6.7**	**6.2**
TOC [ppm]						
TOC [ppm]	65.0					
TC [ppm]	170.0					
IC [ppm]	105.0					
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**5115**	**2451**	**1596**	**710**	**2349**	**3347**

Sampling point: ***Final Effluent - B3***

DATE Time	25-Mar-04	30-Apr-04 13:05	21-May-04 14:10	11-Jun-04 12:05	01-Jul-04 12:45	15-Jul-04 14:11
Site Conditions						
Temperature [°C]	11.3	-	16.8	18.9	18.5	19.4
pH	6.96	-	7.10	7.10	7.05	7.00
COD						
COD [mgL^{-1}]	**26.0**	**16.0**	**70.0**	**24.0**	**32.0**	**33.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-		-	**7.1**	**46.1**
std [+/-]	-	-		-	-	**0.6**
Nitrite						
NO_2-N [mgL^{-1}]	-	-		-	-	**13.9**
std [+/-]	-	-		-	-	**4.7**
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**13.59**	**1.57**	**11.57**	**3.18**	**1.21**	**1.87**
std [+/-]	**2.2**	**0.4**	**8.6**	**0.2**	**0.6**	**0.8**
Soluble Proteins						
Proteins [mgL^{-1}]	**11.2**	**10.1**	**13.0**	**7.9**	**5.9**	**10.9**
std [+/-]	**1.7**	**2.3**	**0.9**	**0.2**	**0.1**	**0.1**
Ratio Soluble Proteins/Carbohydrates						
	0.8	**6.5**	**1.1**	**2.5**	**4.8**	**5.8**
TOC						
TOC [ppm]	4.594	-	-			
TC [ppm]	61.870	-	-			
IC [ppm]	57.280	-	-			
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**104**	**4**	**n.d.**	**12**	**99**	**80**

Sampling point: ***Oxidation Ditch - B2***

DATE Time	25-Mar-04	30-Apr-04 12:55	21-May-04 14:00	11-Jun-04 11:55	01-Jul-04 12:40	15-Jul-04 14:25
Site Conditions						
Temperature	11.3	-	16.9	18.6	18.4	19.2
pH	6.65	-	6.85	6.85	6.68	6.60
COD						
COD [mgL^{-1}]	**28.0**	**26.0**	**74.0**	**76.0**	**78.0**	**49.0**
Nitrate						
NO_3-N [mgL^{-1}]	-	-	-	-	2.6	11.6
std [+/-]	-	-	-	-	-	3.3
Nitrite						
NO_2-N [mgL^{-1}]	-	-	-	-	-	22.2
std [+/-]	-	-	-	-	-	6.1
Soluble Carbohydrates						
Soluble Carbohydrates [mgL^{-1}]	**15.3**	**8.0**	**9.4**	**2.0**	**1.5**	**2.9**
std [+/-]	0.1	2.0	3.9	1.4	0.1	0.3
Soluble Proteins						
Proteins [mgL^{-1}]	**68.5**	**9.9**	**14.1**	**17.6**	**8.8**	**12.8**
std [+/-]	5.4	0.7	0.5	2.2	0.3	0.8
Ratio Soluble Proteins/Carbohydrates						
	4.5	**1.2**	**1.5**	**8.7**	**5.8**	**4.4**
EPS - Extraction						
comments			microcentrifuged	microcentrifuged		
EPS - COD						
COD_{EPS} [mgL^{-1}]			820.00	380.00	730.00	504.00
Extracellular Carbohydrates						
mg EPS-Carbohydrates/g SS	**21.2**		**40.5**	**87.8**	**8.0**	**11.3**
std [+/-]	*0.1*		*0.4*	*24.8*	*0.4*	*0.1*
mg EPS-Carbohydrates/g VSS	**27.8**		**60.5**	**127.5**	**10.2**	**15.3**
std [+/-]	*0.1*		*0.6*	*36.1*	*0.5*	*0.2*
Extracellular Proteins						
mg EPS-Proteins/g SS	**86.3**		**53.0**	**41.0**	**39.3**	**28.3**
std [+/-]	*2.0*		*4.8*	*0.0*	*1.2*	*0.6*
mg EPS-Proteins/g VSS	**113.2**		**79.3**	**59.5**	**50.2**	**38.5**
std [+/-]	*2.6*		*7.2*	*0.0*	*1.5*	*0.8*
Ratio EPS Proteins/Carbohydrates						
	4.1		**1.3**	**0.5**	**4.9**	**2.5**
Total&Volatile Solids per mg/g						
TS [mg/g wet sludge]	**60.2**				**56.0**	**81.3**
VTS [%]	**74.1**				**78.4**	**77.4**
Total&Volatile Solids per mg/L						
TS [mgL^{-1}]		**4.0**	**4.5**	**5.9**	**2.8**	**2.4**
VTS [%]		**69.5**	**68.6**	**67.4**	**85.2**	**67.1**
Suspended & Volatile Solids						
SS [mgL^{-1}]	**2532**	**2590**	**2811**	**3165**	**2234**	**2328**
VSS [%]	**76.2**	**69.1**	**66.9**	**68.8**	**78.2**	**73.6**
Carbon						
TOC [ppm]	71.11					
TC [ppm]	73.03					
IC [ppm]	1.92					
Particle Size	-					
specific surface [m^2/g]	0.069	0.0779	0.0938	0.0714	0.0846	0.0755
d (0.1)	47.061	43.037	37.688	50.176	38.208	43.728
d (0.9)	471.636	383.118	246.633	532.482	376.457	528.439
average diameter d (0.5)	**172.830**	**147.245**	**109.169**	**152.446**	**138.594**	**156.546**
Traced Pharmaceutical						
Triclosan [ngL^{-1}]	**183**	**43**	**177**	**29**	**117**	**47**

Calculation: ***Triclosan load per day and per capita***

Site B PE: 13,440; average flow: 2,700 $m^3d^{-1,}$ *flow 09.09.04 = 2514m³/d; **flow 14.09.04 = 3898m³/d							
date	Triclosan concentration ngL^{-1}		load of Triclosan g/day		Triclosan µg/day per capita		Overall removall rate [%]
	Influent	Final Eflluent	Influent	Final Eflluent	Influent	Final Eflluent	
	Tric B1	Tric B3	Tric B1	Tric B3	Tric B1	Tric B3	
25-Mar-04	5115	104	13.8	0.281	1028	21	*98.0*
30-Apr-04	2451	4	6.6	0.011	492	1	*99.8*
21-May-04	1596		4.3		321		
11-Jun-04	710	12	1.9	0.032	143	2	*98.3*
01-Jul-04	2349	99	6.3	0.267	472	20	*95.8*
15-Jul-04	3347	80	9.0	0.216	672	16	*97.6*
average	*2595*	*60*	*7.0*	*0.162*	*521*	*12*	*97.9*
09-Sep-04	*4328*	*112*	*10.9*	*0.282*	*810*	*21*	*97.4*
14-Sep-04	*1627*	*141*	*6.3*	*0.550*	*472*	*41*	*91.3*
average,**	*2978*	*127*	*9*	*0.416*	*641*	*31*	*94.4*

*, ** *calculated with measured effluent flow rates for specific day*

date	Triclosan concentration ngL-1			load of Triclosan g/day		
	Influent	OD	Final Eflluent	Influent	OD	Final Eflluent
	Tric B1	Tric B2	Tric B3	Tric B1	Tric B2	Tric B3
25-Mar-04	5115	183	104	13.810	0.494	0.281
30-Apr-04	2451	43	4	6.618	0.116	0.011
21-May-04	1596	177		4.309	0.478	
11-Jun-04	710	29	12	1.917	0.078	0.032
01-Jul-04	2349	117	99	6.342	0.316	0.267
15-Jul-04	3347	47	80	9.037	0.127	0.216
09-Sep-04	*4328*	*943*	*112*	*10.9*	*2.371*	*0.282*
14-Sep-04	*1627*	*181*	*141*	*6.3*	*0.706*	*0.550*
average	*2595*	*83*	*60*	*7.407*	*0.586*	*0.234*

Appendix 5.2.4 -2 - Full Scale Sampling List - Site B - 48 hour monitoring

		pH			Soluble COD [mgL^{-1}]				TC [mgL^{-1}]				IC [mgL^{-1}]			
n°	Date	pH_Inf	pH_OD	pH_Eff	COD_Inf	COD_OD	COD_Eff	COD_OR	TC_Inf	TC_OD	TC_Eff	TC_OR	IC_Inf	IC_OD	IC_Eff	IC_remX
1	13/09/2004 10:00	7.30	6.85	7.20	132.00	51.00	34.00	**74.24**	110.70	96.11	75.14	**32.12**	61.97	45.97	42.81	**30.92**
2	13/09/2004 12:00	7.28	6.87	7.23	195.00	36.50	28.00	**85.64**	131.20	80.02	66.51	**49.31**	76.64	47.96	40.15	**47.61**
3	13/09/2004 14:00	7.34	6.84	7.23	107.00	38.00	32.00	**70.09**	90.65	79.98	62.75	**30.78**	58.08	46.82	38.00	**34.57**
4	13/09/2004 16:00	7.26	6.88	7.23	83.00	27.00	24.00	**71.08**	78.61	76.73	58.11	**26.08**	52.19	47.45	27.84	**46.66**
5	13/09/2004 18:00	7.20	6.85	7.18	135.00	33.50	29.00	**78.52**	96.33	79.80	66.30	**31.17**	55.76	47.42	37.80	**32.21**
6	13/09/2004 20:00	6.98	6.90	7.14	146.00	37.00	27.00	**81.51**	90.67	75.34	62.14	**31.47**	53.51	46.67	38.64	**27.79**
7	13/09/2004 22:00	6.56	6.94	7.16	368.00	34.50	27.00	**92.66**	139.30	75.52	59.56	**57.24**	70.01	46.06	38.46	**45.06**
8	14/09/2004 00:00	7.30	6.94	7.18	195.00	37.00	27.00	**86.15**	111.10	76.52	60.93	**45.16**	67.02	46.39	40.03	**40.27**
9	14/09/2004 02:00	7.39	6.97	7.18	181.00	35.00	26.00	**85.64**	103.50	77.36	61.34	**40.73**	64.71	47.43	38.24	**40.91**
x	14/09/2004 04:00	5.86		7.17	374.00		24.00	**93.58**	119.00		60.03	**49.55**	56.51		37.72	**33.25**
10	14/09/2004 06:00	6.55	6.93	7.16	245.00	28.50	25.00	**89.80**	106.60	76.56	65.58	**38.48**	60.19	47.08	39.95	**33.63**
11	14/09/2004 08:00	7.58	6.87	7.10	149.00	37.00	24.00	**83.89**	117.20	75.70	60.48	**48.40**	77.61	45.11	41.16	**46.97**
12	14/09/2004 10:00	7.60	7.04	7.06	165.00	34.00	23.00	**86.06**	116.10	77.47	64.22	**44.69**	74.64	48.43	40.37	**45.91**
13	14/09/2004 12:00	7.13	6.91	7.16	263.00	47.00	24.00	**90.87**	135.70	83.41	59.70	**56.01**	77.64	49.93	37.02	**52.32**
14	14/09/2004 14:00	7.31	6.90	7.19	193.00	39.00	26.00	**86.53**	127.60	93.22	49.54	**61.18**	77.43	49.26	34.28	**55.73**
15	14/09/2004 16:00	7.25	6.92	7.11	120.00	34.00	19.00	**84.17**	90.56	82.49	49.98	**44.81**	57.15	48.66	20.00	**65.00**
16	14/09/2004 18:00	7.02	6.93	7.12	149.00	33.00	24.00	**83.89**	84.79	77.63	49.04	**42.16**	48.71	47.08	33.56	**31.10**
17	14/09/2004 20:00	7.19	6.95	7.05	99.00	35.00	23.00	**76.77**	80.60	81.13	52.40	**34.99**	50.06	47.95	34.94	**30.20**
18	14/09/2004 22:00	6.99	6.93	7.07	198.00	30.00	22.00	**88.89**	117.00	79.71	48.57	**58.49**	69.03	45.75	34.15	**50.53**
19	15/09/2004 00:00	7.06	6.94	7.22	220.00	32.00	25.00	**88.64**	128.10	77.76	63.90	**50.12**	72.12	47.48	36.15	**49.88**
20	15/09/2004 02:00	7.02	6.90	7.10	235.00	31.00	23.00	**90.21**	123.80	76.74	48.84	**60.55**	70.87	47.40	33.41	**52.86**
21	15/09/2004 04:00	6.33	6.93	7.19	386.00	39.00	20.00	**94.82**	153.60	78.83	52.16	**66.04**	87.52	46.97	36.63	**58.15**
22	15/09/2004 06:00	6.93	6.90	7.09	160.00	24.00	20.00	**87.50**	108.50	75.61	51.00	**53.00**	69.30	47.52	36.38	**47.50**
23	15/09/2004 08:00	7.31	6.91	7.01	188.00	19.00	25.00	**86.70**	128.20	78.42	54.85	**57.22**	83.08	47.81	38.17	**54.06**
24	15/09/2004 10:00	7.43	6.88	7.03	163.00	31.00	25.00	**84.66**	125.60	72.47	55.68	**55.67**	82.77	45.18	38.15	**53.91**
24h	09/09/2004	7.45	7.24	7.59	274.00	45.00	33.00	**87.96**								

Appendix 5.2.4 - 2 - continued

		TOC [mgL^{-1}]				Soluble Proteins [mgL^{-1}]						Soluble Carbohydrates [mgL^{-1}]						Ratio Soluble Proteins/Carbs		
n°	Date	TOC_Inf	TOC_OD	TOC_Eff	TOC_rem	P_Inf	std	P_OD	std	P_Eff	std	Carb_Inf	std	Carb_OD	std	Carb_Eff	std	P/C_Inf	P/C_OD	P/C_Eff
1	13/09/2004 10:00	48.73	50.14	32.33	**33.65**	24.03	0.7	7.04	0.7	6.09	1.0	5.99	1.5	3.94	0.1	3.70	0.7	4.0	1.8	1.6
2	13/09/2004 12:00	54.56	32.06	26.36	**51.69**	30.25	0.2	7.10	0.6	4.94	0.7	2.56	0.9	5.51	0.1	4.48	1.8	11.8	1.3	1.1
3	13/09/2004 14:00	32.57	33.16	24.75	**24.01**	14.94	0.4	6.85	0.4	4.88	0.1	3.47	1.3	3.28	0.9	4.58	1.2	4.3	2.1	1.1
4	13/09/2004 16:00	26.42	29.28	30.27	**-14.57**	11.77	0.3	6.42	0.2	4.69	0.3	3.30	2.4	3.48	0.6	5.05	1.8	3.6	1.8	0.9
5	13/09/2004 18:00	40.57	32.38	28.50	**29.75**	21.15	0.4	6.30	0.0	7.65	2.1	2.50	2.1	11.06	0.4	5.82	3.1	8.5	0.6	1.3
6	13/09/2004 20:00	37.16	28.67	23.50	**36.76**	19.92	0.5	5.49	0.6	10.86	8.7	5.82	4.2	5.05	0.3	3.06	0.5	3.4	1.1	3.5
7	13/09/2004 22:00	69.29	29.46	21.10	**69.55**	44.69	1.3	6.42	1.0	4.44	0.2	4.88	1.4	2.73	1.7	4.14	2.6	9.2	2.4	1.1
8	14/09/2004 00:00	44.08	30.13	20.90	**52.59**	25.84	0.7	5.37	0.3	11.23	3.7	7.04	0.2	8.33	4.2	6.40	2.4	3.7	0.6	1.8
9	14/09/2004 02:00	38.79	29.93	23.10	**40.45**	22.22	0.4	5.37	0.4	6.85	0.1	6.90	4.5	0.61	0.3	4.60	0.4	3.2	8.9	1.5
x	14/09/2004 04:00	62.49		22.31	**64.30**	45.64	0.3		0.0	6.17	0.7	7.71	3.7		0.0	2.88	0.4	5.9		2.1
10	14/09/2004 06:00	46.41	29.48	25.63	**44.77**	27.96	0.1	5.68	0.2	5.49	0.6	4.88	2.0	2.53	3.3	4.24	2.0	5.7	2.2	1.3
11	14/09/2004 08:00	39.59	30.59	19.32	**51.20**	22.43	1.0	18.70	11.3	4.81	0.5	4.71	2.0	11.06	5.5	6.33	4.9	4.8	1.7	0.8
12	14/09/2004 10:00	41.46	29.04	23.85	**42.47**	24.12	0.6	4.26	0.3	6.05	1.0	9.66	1.7	7.98	1.6	5.49	2.0	2.5	0.5	1.1
13	14/09/2004 12:00	58.06	33.48	22.68	**60.94**	31.44	0.7	8.95	0.1	7.45	0.8	13.10	1.7	4.95	2.0	4.81	3.2	2.4	1.8	1.5
14	14/09/2004 14:00	50.17	43.96	15.26	**69.58**	30.74	0.7	8.52	0.2	6.95	0.6	11.92	1.2	4.55	1.6	3.43	0.9	2.6	1.9	2.0
15	14/09/2004 16:00	33.41	33.83	29.98	**10.27**	18.44	0.1	8.40	0.2	7.65	0.4	7.17	2.7	4.14	1.9	3.74	1.1	2.6	2.0	2.0
16	14/09/2004 18:00	36.08	30.55	15.48	**57.10**	22.14	0.6	7.65	0.0	5.97	0.3	7.88	1.1	4.80	1.4	3.37	1.1	2.8	1.6	1.8
17	14/09/2004 20:00	30.54	33.18	17.46	**42.83**	16.13	0.8	8.09	0.1	6.34	0.4	6.23	2.1	4.70	3.8	3.13	1.1	2.6	1.7	2.0
18	14/09/2004 22:00	47.97	33.96	14.42	**69.94**	23.62	0.5	8.02	0.0	6.83	0.4	8.28	0.8	2.98	1.4	2.66	0.5	2.9	2.7	2.6
19	15/09/2004 00:00	55.98	30.28	27.75	**50.43**	23.62	0.6	7.10	0.4	9.01	0.9	8.96	1.5	2.37	0.2	4.39	2.8	2.6	3.0	2.1
20	15/09/2004 02:00	52.93	29.34	15.43	**70.85**	25.47	0.3	6.85	0.3	7.00	0.2	6.20	1.9	5.30	0.8	3.57	1.4	4.1	1.3	2.0
21	15/09/2004 04:00	66.08	31.86	15.53	**76.50**	26.01	0.2	7.16	0.2	7.33	0.5	10.37	0.1	3.03	2.1	4.85	1.2	2.5	2.4	1.5
22	15/09/2004 06:00	39.20	28.09	14.62	**62.70**	17.49	0.5	6.67	0.2	7.04	0.0	6.26	0.6	2.98	0.1	2.79	0.9	2.8	2.2	2.5
23	15/09/2004 08:00	45.12	30.61	16.68	**63.03**	24.36	0.1	6.23	0.1	7.16	0.7	5.45	0.8	11.57	3.5	2.76	0.3	4.5	0.5	2.6
24	15/09/2004 10:00	42.83	27.29	17.53	**59.07**	46.58	0.4	6.54	0.2	6.87	0.1	4.98	0.5	6.46	0.4	3.69	0.2	9.3	1.0	1.9
24h	09/09/2004	59.31	13.41	11.99	**79.78**	36.79	1.0	7.41	2.1	10.04	0.2	21.41	9.3	6.77	0.7	5.86	0.2	1.7	1.1	1.7

Appendix 5.2.4 - 2 - continued

		Suspended Solids [mgL^{-1}]			Volatile Suspended Solids [%]			EPS						Proteins				Carbohydrates				
n°	Date	SS_Inf	SS_OD	SS_Eff	VSS_Inf	VSS_OD	VSS_Eff	COD_EPS	ratio COD_{EPS}/COD_{SMP}	Ratio COD_{RS}/COD_{EPS}	TC_EPS	IC_EPS	TOC_EPS	P_EPS_SS	std	P_EPS_VSS	std	C_EPS_SS	std	C_EPS_VSS	std	Ratio P/C EPS
1	13/09/2004 10:00	175.00	2642.22	9.59	80.9	77.1	82.8	247	5	14	134.6	9.8	124.8	42.75	4.0	55.4	5.1	12.7	0.8	16.4	1.0	3.4
2	13/09/2004 12:00	196.33	3061.11	4.38	81.3	76.5	90.5	212	6	18	112.2	17.2	95.0	28.07	0.0	36.7	0.0	10.3	3.0	13.4	3.9	2.7
3	13/09/2004 14:00	192.67	2717.78	5.48	82.3	78.8	71.7	207	5	14	108.5	16.7	91.8	30.80	0.4	39.1	0.5	10.5	1.5	13.3	1.9	2.9
4	13/09/2004 16:00	142.33	2705.56	6.73	84.7	77.7	73.8	247	9	15	125.8	23.5	102.3	40.59	2.0	52.2	2.6	17.9	8.7	23.1	11.2	2.3
5	13/09/2004 18:00	187.67	2021.67	8.14	81.3	77.9	82.3	193	6	14	103.6	14.6	89.0	37.19	0.8	47.7	1.0	13.1	1.7	16.8	2.1	2.8
6	13/09/2004 20:00	207.00	2553.33	6.10	80.6	78.2	96.6	286	8	9	138.6	13.0	125.6	37.28	0.0	47.7	0.0	12.3	3.3	15.7	4.2	3.0
7	13/09/2004 22:00	302.67	2613.33	7.16	87.6	78.3	79.7	218	6	14	103.2	22.3	80.9	31.25	1.9	39.9	2.4	12.9	5.6	16.5	7.1	2.4
8	14/09/2004 00:00	150.00	2582.22	15.49	84.5	79.6	81.2	243	7	11	117.4	22.5	95.0	35.57	0.4	44.7	0.5	15.3	1.2	19.2	1.5	2.3
9	14/09/2004 02:00	197.67	2551.67	12.60	87.5	79.0	81.0	267	8	12	130.9	12.7	118.3	41.37	0.6	52.4	0.8	19.3	8.4	24.4	10.7	2.1
x	14/09/2004 04:00	156.33		12.63	90.4		80.0							0.00	0.0	0.0	0.0		0.0		0.0	
10	14/09/2004 06:00	108.00	2695.00	15.76	89.2	79.2	76.7	283	10	12	126.9	16.8	110.1	40.68	0.2	51.4	0.2	13.8	0.7	17.4	0.9	2.9
11	14/09/2004 08:00	162.33	2301.11	9.71	88.8	78.5	81.7	311	8	10	132.5	24.7	107.9	55.45	1.5	70.7	1.9	29.2	12.1	37.3	15.4	1.9
12	14/09/2004 10:00	267.33	2633.89	17.46	85.0	79.2	57.7	276	8	12	128.6	19.4	109.3	38.11	3.4	48.1	4.3	14.9	3.6	18.8	4.6	2.6
13	14/09/2004 12:00	359.00	2081.11	7.42	91.5	79.0	79.2	282	6	10	122.0	14.8	107.2	48.23	0.6	61.0	0.8	17.3	1.8	21.9	2.2	2.8
14	14/09/2004 14:00	302.33	2462.22	8.71	83.9	77.9	81.0	315	8	9	135.0	15.8	119.2	36.10	0.5	46.3	0.6	17.1	2.1	22.0	2.7	2.1
15	14/09/2004 16:00	214.67	2060.56	7.85	83.9	79.3	80.3	254	7	10	116.9	17.3	99.7	38.46	0.5	48.5	0.7	22.7	8.1	28.7	10.3	1.7
16	14/09/2004 18:00	176.33	2431.67	6.08	82.1	79.2	81.4	325	10	9	143.7	15.9	127.8	50.67	0.1	64.0	0.1	22.2	7.7	28.0	9.8	2.3
17	14/09/2004 20:00	320.33	2374.44	5.14	81.2	78.5	80.0	293	8	10	133.8	22.1	111.7	43.62	0.9	55.6	1.1	19.1	5.2	24.3	6.7	2.3
18	14/09/2004 22:00	210.67	2409.44	6.74	95.1	78.4	78.5	283	9	10	122.7	20.5	102.3	45.14	0.5	57.6	0.7	14.7	2.1	18.8	2.7	3.1
19	15/09/2004 00:00	181.67	2198.33	26.18	86.3	75.7	82.0	327	10	9	140.1	24.2	115.9	59.98	3.5	79.2	4.7	18.8	2.6	24.9	3.4	3.2
20	15/09/2004 02:00	185.67	2520.00	44.38	86.0	77.5	74.5	299	10	9	140.0	29.7	110.3	46.35	1.1	59.8	1.4	16.3	0.8	21.0	1.0	2.8
21	15/09/2004 04:00	200.67	2418.33	10.90	89.8	76.3	80.8	292	7	10	128.7	25.2	103.5	46.56	0.4	61.0	0.5	15.3	0.6	20.1	0.7	3.0
22	15/09/2004 06:00	79.00	2557.78	19.19	91.2	77.3	81.1	366	15	8	142.3	25.4	116.9	59.13	1.2	76.5	1.5	19.4	2.4	25.1	3.1	3.0
23	15/09/2004 08:00	155.67	2441.11	5.65	88.2	78.1	81.8	337	18	9	157.7	35.8	121.9	55.33	0.6	70.9	0.8	17.8	1.5	22.8	1.9	3.1
24	15/09/2004 10:00	195.33	2391.11	8.14	86.7	77.4	82.1	364	12	8	127.1	29.8	97.3	41.05	0.3	53.0	0.3	13.4	0.4	17.3	0.5	3.1
24h	09/09/2004	352.17	2552.22	7.40	84.3	77.9	75.7	356	8	8			123.6	42.81	0.6	55.0	0.7	15.6	0.6	20.0	0.8	2.8

Appendix 5.2.4 - 2 - continued

		sludge	Particle size [µm]						Nitrite				Nitrate			
n°	Date	COD_sldg	d_01	d_05	d_09	sp_surf	D_3_4	D_4_3	No2_Inf	NO2_OD	NO2_Eff	NO2_EPS	NO3_Inf	NO3_OD	NO3_Eff	NO3_EPS
1	13/09/2004 10:00	3390.0	33.9	108.6	302.6	0.0988	60.7	160.5		7.6	0.1	0.2		8.2	0.3	0.3
2	13/09/2004 12:00	3827.5	36.6	111.0	280.5	0.0951	63.1	149.7		5.2	0.9	0.2		12.8	0.2	0.2
3	13/09/2004 14:00	2902.5	40.8	129.7	352.4	0.0841	71.4	175.5		6.4	0.9	0.2		13.2	0.5	0.5
4	13/09/2004 16:00	3747.5	40.6	124.9	322.5	0.0859	69.8	165.3		4.8	0.5	0.1		17.0	0.4	0.4
5	13/09/2004 18:00	2680.0	48.7	159.3	439.5	0.0717	83.7	212.3		4.6	0.0	0.1		18.0	0.3	0.3
6	13/09/2004 20:00	2715.0	38.4	120.2	307.1	0.0903	66.5	161.1		3.8	0.8	0.1		20.5	0.4	0.4
7	13/09/2004 22:00	2945.0	41.4	128.6	332.6	0.0843	71.2	172.5		3.7	0.7	0.0	0.023	20.0	0.3	0.3
8	14/09/2004 00:00	2790.0	39.1	168.6	819.5	0.0776	77.4	310.3		3.5	1.4	0.2	0.105	18.9	0.3	0.3
9	14/09/2004 02:00	3325.0	39.5	152.1	761.5	0.0805	74.5	284.9		1.9	1.1	0.2	0.054	20.1	0.3	0.3
x	14/09/2004 04:00										0.0		0.050		0.0	
10	14/09/2004 06:00	3300.0	47.4	156.3	457.1	0.0725	82.8	217.6	2.1	1.7	0.9	0.2	0.067	19.8	0.3	0.3
11	14/09/2004 08:00	3032.5	37.9	125.1	357.0	0.0906	66.2	171.1	0.4	3.9	0.7	0.1	0.040	27.0	0.3	0.3
12	14/09/2004 10:00	3445.0	40.1	132.5	380.4	0.0852	70.4	183.6	0.4	5.4	1.2	0.2	0.029	40.3	0.4	0.4
13	14/09/2004 12:00	2745.0	39.9	125.4	330.5	0.0865	69.4	162.0	0.0	8.3	1.3	0.1	0.024	10.7	0.2	0.2
14	14/09/2004 14:00	2925.0	57.6	271.2	897.2	0.0537	111.7	387.0	0.0	8.3	1.5	0.1	0.027	12.3	0.2	0.2
15	14/09/2004 16:00	2510.0	40.8	139.7	411.1	0.0821	73.1	194.2	0.0	5.1	1.4	0.1	0.035	14.0	0.3	0.3
16	14/09/2004 18:00	2960.0	42.6	148.6	470.1	0.0781	76.7	216.8	0.0	3.1	1.0	0.1	0.023	13.6	0.3	0.3
17	14/09/2004 20:00	2850.0	48.0	176.1	548.8	0.0685	87.6	252.2	0.0	3.6	0.8	0.1	0.029	19.3	0.3	0.3
18	14/09/2004 22:00	2940.0	46.7	165.5	511.3	0.0717	83.7	236.5	0.0	3.3	0.8	0.1	0.029	24.5	0.4	0.4
19	15/09/2004 00:00	2965.0	34.5	109.3	279.6	0.0989	60.7	151.8	0.0	3.9	1.1	0.1	0.062	19.1	0.3	0.3
20	15/09/2004 02:00	2795.0	60.9	272.8	781.9	0.0521	115.2	360.7	0.0	3.6	0.9	0.1	0.058	19.7	0.2	0.2
21	15/09/2004 04:00	3035.0	40.3	126.7	313.7	0.0868	69.1	160.5	0.0	2.9	0.9	0.0	0.057	22.4	0.2	0.2
22	15/09/2004 06:00	3040.0	49.2	178.9	540.1	0.0678	88.5	249.5	0.2	2.7	0.8	0.1	0.045	22.4	0.3	0.3
23	15/09/2004 08:00	3045.0	46.3	163.9	491.0	0.0725	82.7	229.0	0.0	4.9	0.6	0.0	0.041	21.4	0.2	0.2
24	15/09/2004 10:00	2845.0	52.3	233.6	686.5	0.0605	99.2	315.2	0.0	4.6	0.0	0.0	0.026	36.6	0.3	0.3
24h	09/09/2004	2740.0	48.5	139.9	333.5	0.0832	72.1	191.2	0.0	1.9	0.0	0.1	0.068	4.8	0.3	0.3

Appendix 5.2.4 -2 - continued

		Triclosan concentration [ngL^{-1}]			Triclosan EPS content		Triclosan overall removal
n°	Date	Tric_Inf	Tric_OD	Tric_Eff	*Tric_EPS [ngL^{-1}]*	Tric_EPS [ng gSS^{-1}]	Tric OR [%]
1	13/09/2004 10:00	4119	176	2	100	37.85	99.95
2	13/09/2004 12:00	5277	159	33	274	89.67	99.37
3	13/09/2004 14:00	2046	141	181	249	91.57	91.13
4	13/09/2004 16:00		53	130	324	119.61	
5	13/09/2004 18:00	1173	124	17	479	237.15	98.53
6	13/09/2004 20:00	1392	168	176	366	143.24	87.37
7	13/09/2004 22:00	1106	147	109	256	97.93	90.17
8	14/09/2004 00:00	2115	199	n.d	204	79.10	
9	14/09/2004 02:00	1326	161	8	215	84.35	99.38
x	14/09/2004 04:00	880		98			88.85
10	14/09/2004 06:00	108	264	481	222	82.48	
11	14/09/2004 08:00	1936	171	327	339	147.13	83.09
12	14/09/2004 10:00	1220	183	74	131	49.72	93.95
13	14/09/2004 12:00	1918	162	216	417	200.36	88.75
14	14/09/2004 14:00	2674	240	57	270	109.68	97.88
15	14/09/2004 16:00	2427	165	46	579	281.08	98.11
16	14/09/2004 18:00	651	159	141	610	251.00	78.35
17	14/09/2004 20:00	539	159	106	500	210.38	80.36
18	14/09/2004 22:00	1583	150	46	121	50.19	97.12
19	15/09/2004 00:00	3779	161	95	203	92.43	97.49
20	15/09/2004 02:00	2467	102	48	780	309.49	98.04
21	15/09/2004 04:00	1530	111	871	164	67.66	43.06
22	15/09/2004 06:00	288	138	53	154	60.07	81.57
23	15/09/2004 08:00	1550	91	53	424	173.51	96.56
24	15/09/2004 10:00	1170	43	133	627	262.09	88.63
24h	09/09/2004	4328	943	112	1991.00	780.10	97.41